NON-HORMONAL INTRA-VAGINAL CONTRACEPTIVES FROM A BIODEGRADABLE HYDROGEL DELIVERY SYSTEM (BIORING)

Thesis submitted to the

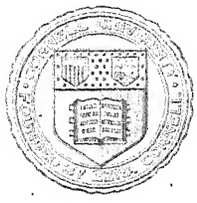
Bundelkhand University
Jhansi 284128, UP
India

For the degree of

Doctor of Philosophy

by

Mukul Singh, MD



Weill Medical College of Cornell University
Dept. of OB/GYN
1300 York Avenue
New York, NY 10021
USA

2005

NON-HORMONAL INTRA-VAGINAL CONTRACEPTIVES FROM A BIODEGRADABLE HYDROGEL DELIVERY SYSTEM (BIORING)

by

Mukul Singh, MD

Weill Medical College of Cornell University
Dept. of OB/GYN
1300 York Avenue
New York, NY 10021
USA

A thesis submitted for the degree of

Doctor of Philosophy

Bundelkhand University
Jhansi 284128, UP
India

2005

Approved by

Ramesh Chandra

Professor Ramesh Chandra, PhD
Vice-Chancellor, Bundelkhand University
Supervisor

Brij B. Saxena

Professor Brij B. Saxena, DSc, DPhil, PhD
Co-Supervisor
Reproductive Endocrinology
Dept. of OB/GYN
Weill Medical College of Cornell University
New York, NY 10021

NON-HORMONAL INTRA-VAGINAL CONTRACEPTIVES FROM A BIODEGRADABLE HYDROGEL DELIVERY SYSTEM (BIORING)

Table of Contents

Acknowledgements

Upon completing my medical education in India, with a specialty in OB/GYN, I pursued my postgraduate training in England under the tutelage of Dr. Patric C. Steptoe. Dr. Steptoe was a pioneer in the field of In Vitro Fertilization (IVF) and fiber optics in the field of OB/GYN. I will always cherish being a part of the team that brought to fruition the birth of the first IVF baby, Louise Brown. I am thankful to Dr. Steptoe for this memorable and unparalleled experience.

This experience intensified my interest in women's reproductive health and propelled me to a career in researching the science of molecular and cellular mechanisms of human reproductive physiology and endocrinology.

Subsequently I began my basic and clinical studies in the field under the guidance of Professor Brij B. Saxena, internationally recognized for his research in the field of reproductive endocrinology at Weill Medical College of Cornell University. USA Dr. Saxena helped me make a

transition from being a clinician to basic scientist. To him, I am thankful for my academic career.

I am also thankful to Professor C.C. Chu of Cornell University, Ithaca for his collaboration in the development of a biodegradable hydrogel based matrix for development of BioRing.

I would also like to thank Dr. Zev Rosenwaks and his team, for providing cervical mucus from patients, Dr Marc Goldstein for providing semen specimen from patients, Dr. William J. Ledger for providing vaginal swabs from patients, and Dr. Michael Bedford for assisting with Rabbit experiments.

I feel fortunate to have met Professor Ramesh Chandra and Dr. Sujata Dass, two very distinguished scientists. I am thankful to them for their support, advice, and encouragement during my research towards development of the BioRing

I wish to thank Rakesh Raghubanshi for his extensive and significant technical as well as typing assistance in the preparation of my thesis work and Mariya Zalesskaya for assisting in typing my thesis. I also want to thank Rebekah Gospin for technical assistance in the laboratory.

Finally I would like to thank my family for their support.

(This work has been protected by a US patent)

Abstract

NON-HORMONAL INTRA-VAGINAL CONTRACEPTIVES FROM A BIODEGRADABLE HYDROGEL DELIVERY SYSTEM (BIORING)

Weill Medical College of Cornell University
Dept. of OB/GYN
1300 York Avenue
New York, NY 10021
USA

Recent studies have shown an increase in the incidence of cervical and breast cancer among women who use hormonal contraceptives for more than five years. There is a need for a non-hormonal contraceptive, which is safe, effective, would by passes the systemic effect, allow women the coital independence and protect against pelvic infections and other sexually transmitted diseases (STDs).

A variety of hormonal and non-hormonal contraceptive methods are available to women today. However, sexually active women still continue to risk unwanted pregnancies and sexually transmitted diseases. We

describe here the development of a novel non-hormonal, biocompatible programmed release intravaginal drug delivery device (BioRing) using a biodegradable hydrogel matrix. The BioRing is composed of a core surrounded by four concentric sheaths composed of dextran, copolymers of polylactide and ε-caprolactone for time release of desired dose. The hydrogel based BioRing was incorporated with ferrous gluconate as a spermiostatic agent, L-ascorbic acid as a reducing agent to increase the viscosity of cervical mucus and mixtures of polyamino-polycarboxylic acid (Ampholines) as the pH modulator to sustain normal vaginal pH (between4-5). Daily eluates from the hydrogel were analyzed for pH levels and spermiostatic activity. In vitro as well as in vivo studies in estrus female rabbits, effect on the cervical mucus and effect on vaginal flora as well as the sterility of the BioRing was evaluated. Eluates, in vitro, up to sixteen days caused complete spermeostasis within 30 seconds, increased the viscosity of the cervical mucus similar to that found in luteal phase of women's menstrual cycle, and sustained the vaginal pH between 4.0 and 5.0. The combined effect of the agents was demonstrated by sperm penetration tests. The eluates did not affect the normal vaginal flora. In vivo studies, the anterior vagina of three estrus female rabbits were instilled with the hydrogel matrix, and after 4 hours the rabbits were inseminated with the semen of a fertile male rabbit. Post-insemination flushes from the vagina of female rabbits were tested for sperm mortality showed that all the sperm were immobile. Addition of

human tubule fluid failed to reanimate the sperms. External genitalia of female rabbit appeared to have no inflammatory reaction. The entire ring was examined for 28 days and no bacterial growth was noted.

The biodegradable hydrogel is being currently developed as an intravaginal ring, for phase I study in humans. The ring is 5.33 cm in diameter and 0.54 cm wide. The flexibility (MTS) maximum load for 1 inch deflection is 1.70N-2.31N to make it easy to insert and remove. Such a device can also be used to deliver anti-HIV as well as other drugs to protect from pelvic infections.

List of Figures

List of Tables

Glossary

Ampholines	A mixture of polyamino & polycarboxyl chains used to adjust the acidity/alkalinity of a solution at a fixed point
Antimicrobicides	Drugs that kill viruses and bacteria
Biocompatible	Compatible/harmless to body physiology
Biodegradable	Denoting a substance that can be chemically decomposed by natural effectors e.g. weather, soil bacteria, plants and animals
Blastocyst	The modified blastula stage of mammalian embryos, which consist of the inner cell mass and a thin trophoblast layer enclosing the blastocele
Buffering Agent	Drives an acidic or alkaline solution to neutral.
Catalytically	One substance enhancing the activity of another substance
Cervical Mucus	Vaginal secretion
Chelate	To combine with a metal in weakly dissociated complexes in which the metal is part of a ring
Copolymer	A polymer in which two or more monomers or base units are combined

Cross-link	To join (adjacent chains of a polymer or protein) by creating covalent bonds
Estrus	That portion or phase of the sexual cycle of female animals characterized by willingness to permit sexual intercourse
Fallopian tube	A tube like organ that facilitates the motility of the egg to the uterus
Fertile	Fruitful, capable of conceiving and bearing young
Fertilization	The act of rendering fertile
Gastric	Abdominal, of, relating to, or associated with the stomach
Hepatic	Of, relating to, or resembling the liver. Acting on or occurring in the liver
HIV	A retrovirus that causes AIDS by infecting helper T- cells of the immune system
Hormone	A substance , usually a peptide or steroid, produced by one tissue and conveyed by the bloodstream to another to effect physiological activity, such as growth or metabolism
Hydrogel	A colloidal gel in which the particles are dispersed in water
Hydrophobic	Incapable of dissolving in water
In Vivo	Within a living organism
Insemination	To introduce or inject semen into the reproductive tract of (a female)
Irradiation	Medical treatment by exposure to radiation
LD	Lethal dose

Lipid Peroxidation	A chemical reaction to generate radicals
Lysis	The destruction of cells, such as blood cells or bacteria, as by the action of a specific lysin that disrupts the cell membrane
Macromer	A larger form of integrated smaller molecules
Matrix	The body substance in which tissue cells are embedded
Multicore-Sheath Design	An inner core surrounded by concentric layers
Neubauer Count Chamber	To count cells and sperm under microscope
PH	A quantitative index of acidity and alkalinity of a solution
Photo-initiator	A chemical reaction initiated by light
Polar	Having a pair of equal and opposite charges
Polymer	A compound, usually of high molecular weight, formed by the combination of simpler molecules
Polysaccharides	Any of a class of carbohydrates, such as starch and cellulose, consisting of a number of monosaccharides joined by glycosidic bonds
Pronuclei	Nucleus of the fertilized egg
Spermicidal	An agent that kills spermatozoa
Spermiostatic	Inhibits the movement of spermatozoa
STDs	Sexually transmitted diseases

Systemic	Relating to or affecting the entire body/an entire organism
Sustained - release	Designed to slowly release a drug in the body over an extended period of time
'Teaser' female	A stimulation to mate
Vaginal flora	A micro- organism in the vagina

Chapter 1: Introduction

1. History and background of contraception

When the modern family planning movement began in the United States and Europe in the early 20th century, its primary purpose was to liberate women from the social and health consequences of unwanted pregnancies. When organized family planning programs reached the Third World, beginning with India in the early 1950s, these programs were viewed as the means to alleviate the pressure of rapid population growth on economic development. In the last decades, the purpose of family planning has broadened to encompass both these objectives and the objective of improving women's health and welfare.

Family planning programs are hypothesized to affect women's lives in at least six areas:

- personal autonomy/self-esteem -- the right to make and stand by one's own decisions; value or regard an individual places on herself;

- health -- both physical and psychological well-being;
- educational attainment -- the ability to obtain an education and the level of educational attainment;
- employment and economic resources -- the nature of employment; acquisition and allocation of resources;
- family relationships -- degree of equality with spouse and role within kinship structure;
- public standing -- ability to participate in public activities and esteem accorded individual women by community.

1.1. History of contraception

From crocodile dung to lactic acid anhydride, contraception dates back as far as ancient Egypt and Greece. Many methods, inventions and substances were used in order to prevent unwanted pregnancies. Oddly enough, using substances such as crocodile dung was never questioned!

How it was used can be left to the imagination.....

Dating back to 1850 BC, ancient Egyptians were responsible for using the infamous dung in addition to the female irrigating her vagina with a mixture of honey and sodium bicarbonate. The Ancient Egyptians also developed a tampon-like object that contained lactic acid anhydride, a chief ingredient in modern contraceptive jellies. We can safely conclude that our ancestors were on the right path! According to an ancient

manuscript called Ebers Papyrus, 1550 BC, women were advised to mix together dates, acacia bark and honey into a sugary paste and place it in the vulva. This method was often efficient because as the sugar ferments it is converted into lactic acid, as mentioned before, a well-known spermicide.

Commonly used and popular among young adults today is the condom. The condom has a rich history and the concept has been known for some time now. Long before condoms came in different sizes, shapes and colors, animal membranes and pieces of linen were sewn together to form a covering for the penis. The rubber condom was developed shortly after the creation of vulcanized rubber in the 1840's, by Goodyear. Vulcanized means that the rubber is subjected to sulfur and extreme heat, which in turn processes the rubber into a strong elastic material. By 1930 liquid latex was used and is still what is used today to manufacture condoms. By the 1990's new technology has improved the quality an effectiveness of the condom enabling manufacturers to make them in different sizes, colors and even flavors.

The origin of the condom is still unknown but it is said that a "Dr. Condom" supplied King Charles II of England with animal tissue sheathes to prevent him from fathering illegitimate children. Public Health concerns started to win over the moralistic attitudes of the time regarding promiscuous sex, when syphilis became rampant among American soldiers in World War 1. By the Second World War, military

leaders had a more realistic attitude about condoms and their use was strongly enforced. Today, condoms are widely used to protect against STD's, (however recent finding tells that infection against HPV virus cannot be protected by condom use) HIV, and pregnancy. The condom's effectiveness can range between 85-98%, depending on how it is used and the concurrent use of spermicide.

After World War II, the increasing rise in world population was alarming. The birth control pill was developed in order to curve this increase. In 1950 an American biologist Gregory Pincus developed the "ideal" oral contraception, which was tested on women from Haiti and Puerto Rico. In 1960 the first oral contraception, Enovid-10, was launched in the US market, known as the "pill". Women were finally enthused about a form of contraception marketed as "safe and effective" and readily used the pill. Within two years oral contraception was used by over 1.2 million women and the numbers continued to rise.

Technology has allowed the contraception industry to flourish, coming up with many different types of contraception for females, with varying degrees of acceptance and success e.g. lower estrogen birth control pills; progestin-releasing intra-uterine device; new ways to deliver spermicides – Vaginal Contraceptive Film, Advantage 24 (bioadhesive gel), Leah's Shield (a fusion of the diaphragm and cervical cap), spermicidal sponge; longer-acting hormonal contraceptives such as Depo-Provera; the female condom; the emergency contraceptive pill (ECP) (called as "morning after

pill); Recently launched and quite innovative is the Evra transdermal contraceptive patch, which is worn on the abdomen area or on the back, a similar idea to the nicotine patch for quitting smoking, it slowly releases estrogen and progestin into the body. Its effectiveness is similar to the oral contraceptive pill and is available from late 2003. Research continues to be done in the areas of contraceptive injections, pills, nasal sprays and implants for men. However there has been little interest in the drug companies developing them, perhaps due to the lack of enthusiasm from men.

There will continue to be research and development in methods of birth control. Though factors such as the high cost of developing drugs, less money for research from governments, and the concern of lawsuits for manufacturers will have a definite influence. The hope is always there for effective, safe and satisfying methods of contraception.

1.2. Contraceptive methods

There are many types of contraceptives in use today much more effective much less harmful to female body but none of which are totally ideal. However each method has been helpful in guiding us to the next step in development of contraceptive technology. Many contraceptives concentrate on keeping sperm out of the uterus, like diaphragms and condoms which provide a physical barrier; spermicides or sponges provide a chemical barrier; Natural Family Planning (NFP) or withdrawal

modifies the sexual act itself. Other methods, generally much more effective, concentrate on interfering with fertility (ovum). The oral contraceptive pill affects the ovum's development and release, an injectable such as Deop-Provera suppresses ovulation or an implant such as Norplant also suppresses ovulation. Intrauterine devices (IUD) help to prevent the fertilized egg from implanting in the uterus.

The major types of contraceptives available today are:

1) Barrier Methods

   a) Male Condoms: Male condoms are thin sheaths made of rubber, vinyl or natural products which are placed on the penis once it is erect. Male condoms may be treated with a spermicide for added protection. Male condoms prevent sperm from gaining access to the female reproductive tract and prevent microorganisms (STDs, except HPV virus including HBV and HIV/AIDS) from passing from one partner to another (latex and vinyl condoms only).

   b) Female Condoms: Female condoms are thin sheaths of polyurethane plastic with polyurethane rings at both ends. They are inserted into the vagina before intercourse. Like male condoms, they prevent sperm from gaining access to the female reproductive tract and prevent microorganisms (STDs, except HPV virus including HBV and HIV/AIDS) from passing from one partner to another through vaginal route.

c) Diaphragms: A diaphragm is a dome-shaped latex (rubber) cup which is inserted into the vagina before intercourse and covers the cervix. Diaphragms prevent sperm from gaining access to the upper reproductive tract (uterus and fallopian tubes) and serve as a holder of spermicide.

d) Spermicides: Spermicides are chemicals (usually nonoxynol-9) that inactivate or kill sperm. They are available as aerosols (foams), creams, vaginal tablets, suppositories, and dissolvable films. Spermicides cause the sperm cell membrane to break, which decreases sperm movement (motility and mobility) and their ability to fertilize the egg. The FDA, however, recently banned nonoxynyl-9 for marketing.

2) Natural Methods

a) Lactational Amenorrhea Method: LAM is the use of breastfeeding as a contraceptive method. It is based on the physiologic effect of suckling to suppress ovulation. To use breastfeeding effectively as a contraceptive requires that the mother either feed the baby nothing but breast milk or, at the very least, breastfeed for almost all feedings. In addition the baby must be less than 6 months old and the mother's menses cannot have returned.

b) Natural Family Planning: To use NFP, a couple voluntarily avoids sexual intercourse during the fertile phase of the woman's cycle

(time when the woman can become pregnant) or has intercourse during the fertile phase to achieve pregnancy. There are four types of NFP: Calendar (Rhythm) Method, Basal Body Temperature, Cervical Mucus Method and Symptothermal Method.

c) Withdrawal (Coitus Interruptus): Withdrawal is a traditional family planning method in which the man completely removes his penis from the woman's vagina before he ejaculates. As a result, sperm do not enter the vagina and fertilization is prevented.

3) Combined (Estrogen/Progestin) Contraceptives

a) Combined Injectable Contraceptives: The two combined injectable contraceptives (CICs), Cyclofem® and Mesigyna®, are injections of the hormones estrogen and progestin which are administered once a month. CICs suppress ovulation, thicken the cervical mucus (preventing sperm penetration) change the endometrium (making implantation less likely), and reduce sperm transport in the upper genital tract (fallopian tubes).

b) Combined Oral Contraceptives: Combined Oral Contraceptives (COCs) are pills, which contain the hormones estrogen and progestin. They are taken daily. COCs suppress ovulation, thicken the cervical mucus (preventing sperm penetration) change the endometrium (making implantation less likely), and reduce sperm transport in the upper genital tract (fallopian tubes). Women above

the age of 35 who smokes cigarettes are not advised to use oral pill. Use of oral pill in smokers increases the risk of Miocardial infarction. (WHO Lancet 1995; 346:1575)

c) Vaginal Ring: NuvaRing is a flexible plastic non biodegradable intravaginal ring about 2.1 inches in diameter it releases a continuous low dose of estrogen and progesterone hormone similar to those available in some oral pill NuvaRing shares similar risks with other hormonal contraceptives, including blood clots, heart attack and stroke. It may also create vaginal discharge and vaginal irritation. NuvaRing, if used correctly, has the same failure rate as the oral pill, however, since it is not a barrier method of contraception or a vehicle for the delivery of microbicite, this ring does not protect against sexually transmitted diseases.

4) Progestin-Only Contraceptives

a) Norplant Implants: The Norplant system consists of six small flexible capsules made of Silastic® tubing which are filled with a synthetic progestin (levonorgestrel). The capsules are inserted just under the skin on the inner side of a woman's upper arm using a minor surgical procedure. Norplant implants work by thickening cervical mucus, changing the endometrium and reducing sperm transport. They provide highly effective contraception for up to 5 years

b) Implenon: Single Rod Effective for two year duration .One of the shortcoming of these Implants. IUDs and injectable is their invasiveness to the human body and need for trained medical professional to deliver them which increases the cost

c) Progestin-Only Injectable Contraceptives: Depo-Provera® and Noristerat® are the two progestin-only injectables contraceptives (PICs). Both are injections of the hormone progestin. They are administered every 3 or 2 months, respectively. PICs work by thickening cervical mucus, changing the endometrium, reducing sperm transport in the upper genital tract and suppressing ovulation.

d) Progestin-Only Pills: Progestin-Only Pills (POPs) contain the hormone progestin. They are taken orally daily by the woman. POPs suppress ovulation, thicken the cervical mucus, change the endometrium, and reduce sperm transport in the upper genital tract.

4) Intrauterine Devices: The intrauterine device (IUD) is a small T-shaped flexible device inserted into the uterine cavity. IUDs can be inert, copper-releasing or progestin-releasing. Copper-releasing IUDs interfere with the ability of sperm to pass through the uterine cavity and with the reproductive process before ova reach the uterine cavity.

Progestin-releasing IUDs also thicken the cervical mucus and change the endometrial lining.

5) Voluntary Sterilization

   a) Tubal Ligation (Female): Tubal ligation is a voluntary surgical procedure for permanently terminating a woman's fertility. Tubal ligation can be done by mini-laparatomy or laparoscopy. Tubal ligation blocks the fallopian tubes (tying and cutting, rings, clips or electrocautery) and sperm are prevented from reaching the ova and causing fertilization.

   b) Male: Vasectomy (Male): Vasectomy is a voluntary surgical procedure for permanently terminating a man's fertility. Vasectomy can be done by the standard method or the no-scalpel technique, which is the preferred method. Vasectomy blocks the vas deferens (ejaculatory duct) so that sperm are not present in the ejaculate.

The table listed below provides a breakdown of contraceptive use, by category, among US women.

Table 1: Contraceptive use among US women

Method	No. of users (in 000s)	% of users
Tubal ligation	10,727	27.7
Pill	10,410	26.9
Male Condom	7,889	20.4

Method	No. of users (in 000s)	% of users
Vasectomy	4,215	10.9
Withdrawal	1,178	3.0
Injectable	1,146	3.0
Periodic Abstinence	883	2.3
Diaphragm	720	1.9
Other	670	1.8
Implant	515	1.3
IUD	310	0.8
TOTAL	38,663	100.0

2. Rationale

Contraceptive technology development has had a great impact on women's reproductive health, however, there are two broad areas still requiring significant continued research and development.

These are:

1) Contraception failure

2) Contraception related adverse events

2.1. Contraception failure

Table 2: Contraceptive failure rates

Contraceptive method	% Failure rate
Male condom:	14%
Withdrawal method:	19%
Diaphragm:	20%
Female condom:	21%
Rhythm method:	25%
Spermicide:	26%
Sponge:	40%
Cervical cap:	40%
No birth control:	85%
Male condom:	14%
Withdrawal method:	19%
Diaphragm:	20%
Female condom:	21%
Rhythm method:	25%
Spermicide:	26%
Sponge:	40%
Cervical cap:	40%
No birth control:	85°%

As is evident from the Table 2 above, the most effective form of birth control is sterilization, with a failure rate of less than 1%, but hormone implants and injections also enjoy a failure rate of less than 1%. Male and female sterilization must be considered irreversible and permanent; therefore appropriate only for people certain they will never desire children. Hormone implants and injections have one advantage over sterilization: they are completely reversible, and therefore most appropriate for young sexually active people. While IUD intrauterine devices have a low failure rate, they may not be appropriate in some cases due to health risks. The progestin birth control pill, once suspected to suffer health risks, has now been proven safe and even beneficial to health, therefore it is appropriate for young sexually active people, because it has a low failure rate and is completely reversible. However, the pill is effective only if women remember to take it every day-hormone implants and injections have an advantage of requiring no daily maintenance. Each method has been helpful in guiding to the next step in contraceptive technology

Table 3: First year contraceptive failure rate in perfect use vs. typical use for various methods.

Method	Perfect Use	Typical Use
No Method	85	85
Implant	0.05	1

Method	Perfect Use	Typical Use
Injectable	0.3	3
Pill (combined)	0.1	8
Diaphragm	6	16
Cervical Cap	18	28
Sponge	15	30
Male Condom	3	15
Spermicides	6	29
Withdrawal	4	27
Periodic Abstinence (calendar method)	9	25
IUD (Copper-T 380A)	0.6	1
Tubal Sterilization	0.5	0.7
Vasectomy	0.1	0.2

2.2. Contraception related adverse events

By providing daily hormones to the woman's body, hormonal methods interfere with normal endocrine and metabolic processes and thus come with a variety of potential serious health risks such as heart disease, stroke, various thromboembolisms, genital cancers, and other physical side effects like weight gain, acne, mood changes, nausea, amenorrhea, and delayed reversal of fertility.

Furthermore, in a discussion of the risks of intercourse due to an exchange in bodily fluids, the topic of sexually transmitted diseases is very relevant. Sexual relations can result in conception as well as infection, in the form of STDs and HIV. New technology is needed that will protect women against both. However, to avoid STDs a woman currently would need to use a barrier method in addition to the most highly effective birth control method available, i.e. hormones. As a result, a woman, who is not in a monogamous relationship with an STD-free partner, would need to end up using two forms of birth control.

2.3. Future contraceptives

Contraceptives prevent unwanted pregnancies and provide better family planning and health care. Convenience, safety, efficacy, and cost as well as the quality of life are usually the concerns in choosing a contraceptive, and these very factors motivate the development of newer and better contraceptives. There is a pressing need to develop a non-hormonal, biocompatible, non-invasive, cost-effective, biodegradable, and convenient barrier device to prevent pregnancy and infection without interfering with sexual relations. The success rate of a contraceptive depends not only upon the efficacy of the contraceptive method, but also upon the users' preference, reversibility, convenience, and compliance. Besides pregnancy, sexual relations can also result in infection. It is thus beneficial that the design of newer contraceptive devices should also

consider the option of protecting women not only against pregnancy but also against transmission of sexually transmitted diseases (STDs). In the future, effective and emerging new antimicrobial (STDs) and antiviral (HIV) agents, which are benign to the vaginal mucosa yet effective as virucides, could possibly be incorporated in this delivery system. The public health significance of developing dual protection for women with a single and reliable device can hardly be exaggerated. Women are the fastest-growing AIDS group, in part because increasingly the primary transmission of HIV is through heterosexual sex, making women particularly vulnerable.

A novel approach for the delivery of birth control through vaginal route arose from the observations of Elsimar Coutinho, MD, who studied the effects of inserting a birth control pill into a woman's vagina. He found that it was absorbed just as efficiently vaginally as orally. Additionally, to cause a contraceptive effect, the hormones avoided the hepatic and gastric pathways and were directly absorbed into the blood circulation, thus reducing some of the systemic side effects.

Further research indicated that vaginal application would require lower levels of hormones rather than oral administration. Thus, in order to avoid the need for daily upkeep, as in oral pill, the need for the development of a long-term vaginal delivery system was identified. Except for barrier methods, surgical procedures, and some IUDs, all birth control methods rely on using hormones to systemically affect the

reproductive function The hormonal class of non-barrier contraceptives are taken orally (e.g., the Pill), implanted (e.g., Norplant, Implanon), or injected (e.g., Depo-Provera) and are based on the understanding that by supplying extra hormones to a woman's body, the uterus is tricked into not developing a thick, nurturing endometrial lining to which a: fertilized egg can attach. Although, these contraceptive devices do not depend on the lack of proper usage since they are directly inserted, injected, or implanted by health care professionals, they are not without drawbacks. Long-term hormonal contraception use and associated problems such as heart disease, stroke, various cancers, and other physical side effects like weight gain, acne, mood changes, and nausea are well documented.

The new contraceptive devices could be free of hormones and should allow women the freedom to use it by themselves, in the privacy of their own home thus enhancing the quality of life.

One of the recommendations made by the Committee on Contraceptive Research and Development in 1996 was to identify agents that are spermiostatic rather than spermicidal, modify mucus secretions from cervical epithelial cells to prevent sperm penetration and are anti-microbial and anti-viral. With current interest in the delivery of steroids, as a contraception method and hormone replacement therapy (HRT) for post-menopause, via non-biodegradable, hormonal intravaginal rings it is innovative to create a biodegradable, non-hormonal and biocompatible intravaginal ring that acts locally and avoids a systemic route to deliver

contraceptive agents. The new device could also carry anti-STD agents (Although the N9 spermicide is banned by FDA for human use, many women depend on them). Thus the development of BioRing is timely and has innovation and significance for womens' health care.

Future development of novel contraceptive methods, which in addition to providing contraception could also incorporate drugs against diseases, would provide a major breakthrough in contraceptive development. Along the lines of this development we propose the following:

1) BioRing: As a response to the growing need for a non-hormonal yet equally effective contraceptive system, we have developed a delivery system, namely BioRing, which truly is the birth control for the new millennium. The BioRing can deliver effective contraception as well as anti STD/HIV medication.

   A Non-hormonal, biodegradable, biocompatible hydrogel based intravaginal delivery system acts locally to minimize adverse metabolic effect. For contraception Iron II, Ascorbic acid, and Ampholines are delivered through the device.

   - Barrier
   - Local (intravaginal, discrete)
   - Biodegradable
   - Biocompatible

- Non-hormonal

Delivery of anti-STD and anti-viral (HIV) agents

- Delivery of drugs for pelvic infection/ diseases
- Duration: 1 to 28 days

2) Contraceptive Vaccine: made up of Lutropin Receptor and hCG-beta based antigen

- Immunocontraceptive
- Systemic
- Duration one year

2.3.1. The BioRing

The development of a novel protection mechanism, which incorporates a chemical barrier, against STD and HIV, in addition to providing contraception, is a major leap in contraceptive technology development.

The intravaginal, non-hormonal, contraceptive ring, BioRing, provides such a solution. BioRing upon insertion will begin to release a steady and safe flow of contraceptive agents to prevent pregnancy on a monthly basis, month after month. BioRing will allow women the freedom to be spontaneous because they no longer have to worry about their birth control on a daily basis and yet they can count on effective month long

protection. In addition, BioRing can provide a safe non-hormonal effective form of birth control, and protection against STDs.

Some other advantages of the BioRing are:

1) Non-systemic: By acting locally, the intravaginal device avoids the systemic route and thus eliminates undesirable systemic effects.

2) Non-hormonal: Instead of being invasive, for example by overpowering the woman's cycle with hormones, the intravaginal device moves in the direction of a natural and gentle form of contraception.

3) Biocompatible: The proposed ring is currently made of hydrogel, which has high water content similar to body tissues, adjustable to the contours of the inner vagina, causes no discomfort, and would not interfere with sexual relations. This hydrogel vastly used to make medical implants and wound closure but never previously used as drug delivery system. All the ingredients used in this device are FDA approved for human use, are inexpensive and since women can use it themselves in the privacy of their own home without going to the physicians office brings the cost further down.

4) Biodegradable: The hydrogel, made of natural polysaccharide and synthetic biodegradable polymer, is hydrolytic, i.e. bioerodes slowly when in contact with water. The body easily absorbs the other ingredients.

3. Literature review

Saxena BB and **Singh M.** Potential of LH-Receptor as an Immuno-contraceptive Vaccine. In `Contraception for Today and Nineties', Edit: Talwar GP, Springer-Verlag, Progress in Vaccinology, Vol. 1, Chapter 22, 239, 1986

Peters RK, Thomas D, Hagan D, Mack T, and Henderson BE. Risk factors for invasive cervical cancer among Latinas and non-Latinas in Los Angeles County. J Natl Cancer Inst 77 (1986), pp. 1063-1077.

Brinton LA, Huggins GR, Lehman HF et al., Long-term use of oral contraceptives and risk of invasive cervical cancer. Int J Cancer 38 (1986), pp. 399-444.

Ebeling K, Nischan P, and Schindler C. Use of oral contraceptives and risk of invasive cervical cancer in previously screened women. Int J Cancer 39 (1987), pp. 427-430.

Saxena BB, **Singh M.** Potential of LH-Receptor as an Immunocontraceptive vaccine: Studies in the Baboon (Papio). In: Contraception Research for Today and the nineties, Volume 1: Progress in Birth Control Vaccines. (Ed.) Talwar, GP, Springer-Verlag Publishers, New York. pp 241-252, 1988.

Bera V, Hannaford P, and Kay C. Oral contraceptive use and malignancies of the genital tract: results from the Royal College of General Practitioners' Oral Contraception Study. Lancet 2 (1988), pp. 1331-1335.

Irwin KL, Rosero-Bixby L, Oberle MW, et al., Oral contraceptives and cervical cancer risk in Costa Rica: detection bias or causal association? JAMA 259 (1988), pp. 59-64.

Oberle MW, Rosero-Bixby L, Irwin KL, et al., Cervical cancer risk and use of DMPA in Costa Rica. Int J Epidemiol 17 (1988), pp. 718-723.

Singh M, Saxena BB, Graver R, Ledger WJ, Contraceptive efficacy of Norethindrone encapsulated in Injectable bio-degradable poly-dl-lactide-co-glycolide micro spheres, Phase II clinical study, Fertility and Sterility, 1989, 55. 973-980.

Cox DR and Snell EJ. In: Analysis of binary data, Chapman and Hall, London (1989), p. 60.

Thomas DB, et al. Monthly injectable contraceptives and cervical carcinoma. American Journal of Epidemiology 130(2):237-247(1989).

Parazzini F. et al. Barrier methods of contraception and the risk of cervical neoplasia. Contraception 40:519-530 (1989).Brinton LA, Reeves WC, Brenes MM, et al. Oral contraceptive use and risk of invasive cervical cancer. Int J Epidemiol 19 (1990), pp. 4-11.

Herrero R, Brinton LA, Reeves WC, et al. Injectable contraceptives and risk of invasive cervical cancer: evidence of an association. Int J Cancer 46 (1990), pp. 5-7.

Herrero R, et al. Risk factors for invasive carcinoma of the uterine cervix in Latin America. Bulletin of the Pan American Health Organization 24:263-283 (1990).

Jones CJ, Brinton LA, Hamman RF, et al. Risk factors for in situ cervical cancer: results from a case-control study. Cancer Res 50 (1990), pp. 3657-3662.

Kjaer SK, et al. Risk factors for cervical human papillomavirus and herpes simplex virus infections in Greenland and Denmark: a population-based study. American Journal of Epidemiology 131:669-682 (1990).

Hildesheim A, et al. Association of oral contraceptive use and human papilloma viruses in invasive cervical cancers. International Journal of Cancer 45:860-864 (1990).

Pal, R, Singh OM, Talwar GP, **Singh M,** Saxena BB. Active immunization of Baboons (Papio anubis) with the bovine LH-receptor. Journal of Reproductive Immunology, 1992, 22, 87-103

World Health Organization. Collaborative Study of Neoplasia and Steroid Contraceptives. Depot-Medroxyprogesterone Acetate (DMPA) and risk of invasive squamous cell cervical cancer. Contraception 45:299-312 (1992).

World Health Organization. Oral contraceptives and neoplasia. Report of a WHO Scientific Group. World Health Organization Technical Report Series 817. Geneva: World Health Organization, 1992.

World Health Organization. Depot-medroxyprogesterone acetate (DMPA) and risk of invasive squamous cell cervical cancer. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Contraception 45 (1992). pp. 299-312.

Coker AL, et al. Barrier methods of contraception and cervical intraepithelial neoplasia. Contraception 45:1-10 (1992).

Bosch FX, Munoz N, de Sanjose S, et al. Risk factors for cervical cancer in Colombia and Spain. Int J Cancer 52 (1992), pp. 750-758.

Munoz N, Bosch FX, de Sanjose S, et al. The causal link between human papillomavirus and invasive cervical cancer: a population-based case-control study in Colombia and Spain. Int J Cancer 52 (1992), pp. 743-749.

Kjaer SK, Engholm G, Dahl C, Bock JE, Lynge E, and Jensen OM. Case-control study of risk factors for cervical squamous-cell neoplasia in Denmark. III. Role of oral contrяcentive use- Cancer Causes Control. 1993 Nov; 4(6):513-9.

Bosch FX, Munoz N, de Sanjose S, et al. Human papillomavirus and cervical intraepithelial neoplasia grade III/carcinoma in situ: a case-control study in Spain and Colombia. Cancer Epidemiol Biomarlers Риev 2 (1993), pp. 415-422.

World Health Organization. Invasive squamous-cell cervical carcinoma and combined oral contraceptives: results from a multinational study. WHO Collaborative Study of Neoplasia and Steroid Contraceptives. IntJCancer 55 (1993), pp. 228-236.

World Health Organization. Collaborative Study of Neoplasia and Steroid Contraceptives. Invasive squamous cell carcinoma and combined oral contraceptives: results from a multinational study. International Journal of Cancer 55:228-236 (1993).

Munoz N, Bosch FX, de Sanjose S, et al. Risk factors for cervical intraepithelial neoplasia grade III/carcinoma in situ in Spain and Colombia. Cancer Epidemiol Biomarkeиs Prev 2 (1993), pp. 423-431.

Eluf-Neto J, Booth M, Munoz N, Bosch FX, Meijer CJ, and Walboomers JM. Human papillomavirus and invasive cervical cancer in Brazil. Br J Cancer 69 (1994), pp. 114-119.

Daling JR, et al. Risk of breast cancer among young women: relationship to induced abortion. Journal of the National Cancer Institute 86:1584-1592 (1994).

Rosenberg L. Induced abortion and breast cancer: more scientific data are needed. Journal of the National Cancer Institute 86:1569-1570 (1994).

Ursin G, Peters RK, Henderson BE, d'Ablaing 3rd G., Monroe KR, and Pike MC. Oral contraceptive use and adenocarcinoma of cervix. Lancet 344 (1994), pp. 1390-1394.

Lazcano-Ponce EC, Hernandez-Avila M, Lopez-Carrillo L, et al. Reproductive risk factors and sexual history associated with cervical cancer in Mexico. Revista de Investigacion Clinica 47 (1995), pp. 377-385 (in Spanish).

Singh M, Lin S, Saxena BB. Effect of Immunization with Lutropin-Receptor on the ovarian Function of Rabbits. Journal of Radioimmunoassay, 16, 1-16, 1995

Ye Z, et al. Combined oral contraceptives and risk of cervical carcinoma in situ. International Journal ofEpidemiology 24(1): 19-26 (February 1995).

Thomas DB, and Ray R. Depot-medroxyprogesterone acetate (DMPA) and risk of invasive adenocarcinomas and adenosquamous carcinomas of the uterine cervix. WHO Collaborative Study of tieoplasia and Steroid Contraceptives. Contraception 52 (1995), pp. 307-312.

Thomas DB, Ye Z, and Ray RM. Cervical carcinoma in situ and use of depot-medroxvprogesterone acetate (DMPA). WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Contraception 51 (1995), pp. 25-31.

Ye Z, Thomas DB, and Ray RM. Combined oral contraceptives and risk of cervical carcinoma in situ. WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Int J Epidemiol 24 (1995), pp. 19-26.

Thomas DB, Ray RM, and WHO. Depot-Medroxyprogesterone Acetate (DMPA) and risk of invasive adenocarcinomas and adenosquamous carcinomas of the uterine cervix. Contraception 52:307-312 (1995).

Thomas DB, et al. Cervical carcinoma in situ and use of Depo-Medroxyprogesterone Acetate (DMPA). Contraception 51:25-31 (1995).

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Human Papillomaviruses. IARC Monogr Eval Carcinog Risks Hum 64 (1995), pp. 1-409.

Singh, M., Thomas, D., Singh, R., Saxena, B.B., Ledger, W.J. A triphasic oral contraceptive pill, CTR-05: Clinical efficacy and safety. The European Journal of Contraception and Reproductive Health Care. Vol. 1(3): 285-92; December 1996

Thomas DB and Ray RM. Oral contraceptives and invasive adenocarcinomas and adenosquamous carcinomas of the uterine cervix. The World Health Organization Collaborative Study of Neoplasia and Steroid Contraceptives. American Journal of Epidemiology 144(3): 281-289 (1996).

Author S. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and hormonal contraceptives: further results. Contraception 54 suppl (1996), pp. 15-1065.

Cuzick J, Sasieni P, and Singer A. Risk factors for invasive cervix cancer in young women. Eur J Cancer 32A (1996), pp. 836-841.

Daling JR, Madeleine MM, B. McKnight, et al. The relationship of human papillomavirus-related cervical tumors to cigarette smoking, oral contraceptive use, and prior herpes simplex virus type 2 infection. Cancer Epidemiol Biomarkers Prev 5 (1996), pp. 541-548.

Zondervan KT, Carpenter LM, Painter R, MP Vessey. Oral contraceptives and cervical cancer: further findings from the Oxford Family Planning Association contraceptive study. Br J Cancer 73 (1996), pp. 1291-1297.

Zondervan KT, et al. Oral contraceptives and cervical cancer-http://www.path.orgloutlook/html/ 15_1.hhn 5/27/2003

Further findings from the Oxford Family Planning Association contraceptive study. British Journal of Cancer 73:1291-1297 (1996).

Rookus MA and Leeuwen FE. Induced abortion and risk for breast cancer: reporting (recall) bias in a Dutch case-control study. Journal of the National Cancer Institute 88:1759-1764 (1996).

Singh M, Saxena BB, Raghubanshi RR, Ledger WJ, Harman SM, Leonard RJ. Biodegradable Norethindrone (NET)- Cholesterol Contraceptive Implants: Phase IIA: A clinical study in women. Contraception, Vol. 55: 23-33; January, 1997.

Singh M, Saxena BB, Singh R, Kaplan J, Ledger WJ. Contraceptive efficacy of Norethindrone encapsulated in injectable biodegradable poly-dl-lactide-co-glycolide micro spheres (NET-90): phase III clinical study. Advances in Contraception, 13: 1-11; 1997.

Melbye M, et al. Induced abortion and the risk of breast cancer. New England Journal of Medicine 336:81-85 (1997).

Sherris J, et al. Planning Appropriate Cervical Cancer Control Programs. Seattle: PATH (March 1997).

Henshaw SK. Unintended pregnancy in the United States. Fam Plan Perspect, 30(1), 24. (1998)

Ferlay J, Parkin DM, and Pisani P. GLOBOCAN Yol 1: cancer incidence and mortality worldwide (IARC cancerbase number 3), IARC, Lyon (1998).

Parazzini F, Chatenoud L, La Vecchia F. Chiaffarino E. Ricci and E. Negri C. Time since last use of oral contraceptives and risk of invasive cervical cancer. Eur J Cancer 34 (1998), pp. 884-888.

Chaouki N, Bosch FX, Munoz N, et al. The viral origin of cervical cancer in Rabat, Morocco. Int J Cancer 75 (1998), pp. 546-554.

Chichareon S, Herrero R, Munoz N, et al. Risk factors for cervical cancer in Thailand: a case-control study. J Natl Cancer Inst 90 (1998), pp. 50-57.

Ngelangel C, Munoz N, Bosch FX, et al. Causes of cervical cancer in the Philippines: a case-control study. J Natl Cancer Inst 90 (1998), pp. 43-49.

Lacey JV, Brinton L, Abbas FM, et al. Oral contraceptives as risk factors for cervical adenocarcinomas and squamous cell carcinomas. Cancer Epidemiol Biomarkers Prev 8 (1999), pp. 1079-1085.

Ronnerdag, M, and Odlind V. Health effects of long-term use of the intrauterine levonogestrel-releasing system. Acta Obstet Gynecol Scand, 78(8), 716. (1999).

Walboomers JM, Jacobs MV, Manos M, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189 (1999), pp. 12-19.

Kaunitz AM. Comparative safety, efficacy and cycle control of Lunelle monthly contraceptive injection (medroxyprogesterone acetate and estradiol cypionate injectable suspension) and Ortho Nowm 7/7/7 oral contraceptive (northendrone ethinyl estradiol triphasic). Contraception, 60(4), 179. (1999).

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Hormonal contraception and post-menopausal hormonal therapy. Lyon, International Agency for Research on Cancer. IARC Monogr Eval Carcinog Risks Hum 1999; 72: 49-66 and 339-341.

Ylitalo N, Sorensen P, Josefsson A, et al. Smoking and oral contraceptives as risk factors for cervical carcinoma in situ. Int.I Cancer 81 (1999), pp. 357-365.

Deacon J, Evans CD, Yule R, et al. Sexual behavior and smoking as determinants of cervical HPV infection and of CIN3 among those infected: a case-control study nested within the Manchester cohort. Br J Cancer 83 (2000), pp. 1565-1572.

Kaunitz AM. Efficacy, cycle control, and safety of two triphasic oral contraceptives: Cyclessa (desogestrel/ethinyl estradiol) and Ortho-Novum 717/7 (northendrone/ethinyl estradiol): A randomized clinical trial. Contraception, 61(5), 295. (2000).

Rolon PA, Smith JS, Munoz N, et al. Human papillomavirus infection and invasive cervical cancer in Paraguay. Int J Cancer 85 (2000), pp. 486-491.

Krattenmacher R. Drospirenone: Pharmacology and pharmacokinetics of a unique progesten. Contraception, 62(1-29). (2000).

Johansson ED. The return of the pharmaceutical industry to the market of contraception. Steroids, 65(10-11), 709. (2000).

Timmer C and Mulders T. Pharmacokinetics of etonogestrel and ethinylestradiol released from a combined contraceptive vaginal ring. Clin Pharmacokinet, 39(3), 233. (2000).

Cady P. Family Planning. July 25, 2000.

Van Damme L. Clinical research with topical microbicides as a potential HIV prevention method. Aids Reader. September 2000, 552.

Mulders TM and Dieben TM. Use of the novel combined contraceptive vaginal ring NuvaRing for ovulation inhibition. Fertil Steril, 75(5), 865. (2001).

Santos C, Munoz N, Klug SJ, et al., HPV types and cofactors causing cervical cancer in Peru. Br J Cancer 85 (2001), pp. 966-971.

Hildesheim A, Herrero R, Castle P, et al. HPV co-factors related to the development of cervical cancer: results from a population-based study in Costa Rica. Br J Cancer 84 (2001), pp. 1219-1226.

Madeleine MM, Daling JR, Schwartz SM, et al. Human papillomavirus and long-term oral contraceptive use increase the risk of adenocarcinoma in situ of the cervix. Cancer Epidemiol Biomarkers Prev 10 (2001), pp. 171-177.

Zinger M and Thomas MA. Using the levonorgestrel IUS. Contemporary OB/GYN, 46(5), 35. (2001).

Audet MC, Moreau M, et al. Evaluation of contraceptive efficacy and cycle control of a transdermal contraceptive patch vs. an oral contraceptive. JAMA, 285(18), 2347. (2001).

Roumen FJ, Apter D, et al. Efficacy, tolerability, and acceptability of a novel contraceptive vaginal ring releasing etonogestrel and ethinyl oestrodiol. Hum Reprod, 16(3), 469. (2001).

Ledger WJ, Gee R, **Singh M**, Witkins SS. Human Papilloma Virus Testing. The 7th Annual Meeting International Infections Disease Society, Pasadena, California, May3-5, 2002.

Bjarnadottir RL, Tuppurainen M, and Killick SR. Comparison of cycle control with a combined contraceptive vaginal ring and oral levonorgestrel/ethinyl estradiol. Am J Obstet Gynecol, 186(3), 389 (2002).

Mishell D, Burkrnan R, and Zieman M. Transdermal contraception: A new technology for women. The Female Patient, Supplement, 3. (2002, August).

Zieman M, Abrams L, et al. The introduction of a transdermal hormonal contraceptive (Ortho Evra/Evra). Fertil Steril, 77(2, Supp12), S1. (2002).

Hubacher D and Grimes D. Noncontraceptive health benefits of intrauterine devices: A systematic review. Obstet Gynecol Supp, 57(2), 120. (2002).

Berrington A, Jha PK, Peto J, Green J, and Hermon C. Oral contraceptives and cervical cancer. Lancet 360 (2002), p. 410.

Moreno V, Bosch FX, Munoz N, et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. The Lancet 2002; 359: 1085-92.

Munoz N, Franceschi S, Bosetti C, et al. Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case-control study. The Lancet 2002; 359: 1093-101.

Moreno V, Bosch FX, Munoz N, et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. Lancet 359 (2002), pp. 1085-1092.

Skegg DC. Oral contraceptives, parity, and cervical cancer. Lancet 359 (2002), pp. 1080-1081.

Brown C, Frank L, and Wan 7. A new monophasic oral contraceptive containing drospirenone. Effect on premenstrual symptoms. J Reprod Med, 47(1), (2002).

Van Vloten W, van Haselen C, et al. The effect of two combined oral contraceptives containing either drospirenone or cyproterone acetate on acne and seborrhea. Cutis, 69(4S), 2. (2002).

Ryan CC, and Futterman D. "Counseling clients for the new monthly injectable-Lunelle." Health & Sexuality. 2001. www.arh .orhealthcareproviders/onlinepublications/healthandsexuality /ndic/counceling.cfm?ID= 213 (18 Nov. 2002).

Saxena BB, Clavio A, **Singh M**, Rathnam P, et al. Modulation of Overian Function in Female Dog immunization with bovine Luteinizing hormone receptor Reproductive Dom Anim 37,9-17(2002)

Arkin ES, Einhaus KB, **Singh M**, Thompson MM, Alexander NJ. Results of a patient acceptance survey administered to women in NuvaRing® Premier Program™ 51st Annual Clinical Meeting. The American College of Obstetrics and Gynecology. New Orleans. April 26-30, 2003 Poster Presentation poster number 1734.

Saxena BB, Clavio A, **Singh M**, Rathnam P, et al. Effect of immunization with bovine Luteinizing hormone receptor on ovarian function in cats. AJVR,Vol 64, No 3, March 2003

Berrington A, Cox DR. Generalized least squares for the synthesis of correlated information. Biostatistics (in press).

Green J, Berrington A, Gonzalez A, Smith JS, et al. Human papillomavirus and use of oral contraceptives. Br J Cancer (in press).

Effect of Non-Hormonal Intravaginal Contraceptive from a Biodegradable Hydrogel delivery system: BioRing. **Faculty speaker: Mukul Singh, MD. Chief Resident's Day**. Dept. Of OB/GYN, Weill Medical College of Cornell University June, 2004

Presenting Author: **Mukul Singh, MD.** Co Authors: Saxena BB, Dass S, Gospin RM, Chu, CC, Ledger WJ. "Non-Hormonal Intravaginal Contraceptives Device: BioRing" International Conference on: Chemistry Biology Interface Synergistic New Frontiers (CBSINF), in New Delhi, India, November 21-26, 2004.

Chapter 2: Development of the BioRing

1. Introduction

The midcycle or periovulatory period in normally menstruating women is the fertile period and is characterized by the rapid increase and decline in levels of estrogen and the beginning in the rise of progesterone. The midcycle fertile phase also shows a pronounced secretion of copious and non-viscous cervical mucus that facilitates sperm penetration and migration during preovulatory period. Toward the development of a biodegradable, intravaginal contraceptive delivery device; it was proposed, first, to reduce sperm motility to zero (spermiostatic effect); and therefore to find reagents second, to increase the viscosity of cervical mucus to impede the sperm motility, and third, to sustain a pH around 5 in the vaginal cavity to augment the total spermiostatic effect.

The following specific aims were proposed to achieve optimum ingredient selection:

1) To develop and synthesize biodegradable core sheath matrices having a wide range of compositions, and configurations as the delivery vehicle for incorporating various spermiostatic agents to achieve efficacious daily release rate for extended period of contraception, and in vivo and in vitro testing

2) To identify and evaluate the efficacy of various concentrations of non-hormonal and biocompatible inorganic salts and their derivatives for their spermiostatic effect on human and rabbit sermatozoa in vitro.

3) To identify and evaluate the efficacy of reducing agents as L-ascorbic acid to increase the viscocity of the cervical mucus to inhibit the sperm migration and mortality.

4) To identify and evaluate non hormonal and biocompatible agent like polyamino polycarboxilic acid mixture to buffer and provide an acidic pH environment of the vaginal cavity to approximately 4.5-6 to enhance the spermiostatic effect and restore normal vaginal flora.

5) To construct the intravaginal biodegradable device in the form of a ring.

2. Material selection

2.1. Hydrogel

Although, a controlled release biodegradable delivery vehicle of bioactive agents for contraception over extended periods has not been developed thus far, hydrogels as delivery vehicles have received significant attention for use as medical implants and wound closure. Hydrogels are of special interest in biological environments since they have a high water content as found in body tissues, and are highly biocompatible. Hydrogels and natural biological gels have hydrodynamic properties similar to that of cells and tissues in the body. Hydrogels minimize mechanical and frictional irritation to the surrounding tissue because of their soft and compliant nature. Therefore, hydrogels provide a far more user-friendly delivery vehicle than the relatively hydrophobic carriers like silicone, vinyl acetate, etc. Biodegradable hydrogels are made up of natural polysaccharides e.g. dextran and synthetic, biodegradable, hydrophobic polymers e.g. poly-DL-lactide.

Biodegradable hydrogels as a delivery vehicle have the advantage of being environmentally friendly to the human body (due to their biodegradability) and of providing more predictable, controlled release of the impregnated drugs. Recently, two new classes of biodegradable hydrogels have been developed for more controlled release of a wide range of bioactive agents e.g., Ferrous Gluconate (Iron II), Sodium

ascorbate (L-ascorbic acid), Polyamino Polycarboxilic acid (Ampholines) as well as substrates for tissue engineering and regeneration. These new biodegradable hydrogels are synthesized from a naturally occurring biodegradable, biocompatible and hydrophilic polysaccharide (i.e., dextran) and synthetic biodegradable hydrophobic polymers (i.e., polylactide). Dextran consists primarily of 1,6-α-D-glucopyranosyl residues and has three hydroxyl groups per glucose residue that could provide greater flexibility in the formulation of hydrogrls. Dextran has been widely used for many biomedical purposes, such as plasma expander and controlled drug delivery vehicle, because of its highly hydrophilic nature and biocompatibility. It is also possible to incorporate dextranase in order to facilitate biodegradation of dextran for the meeting of specific clinical needs. Both dexran and synthetic biodegradable polyesters like polyglycolide (PGA), polylactide (PLA) or their copolymers are FDA approved for human use raw biomaterials, which are commercially most successful as synthetic, absorbable polymers for biomedical use in the form of medical implants and wound closure devices. The degradation products of PGA and PLA are natural metabolites and are readily eliminated by the human body.

These new biodegradable hydrogels described here have a unique three-dimensional porous network structure. By controlling the ratio of polysaccharide (e.g., dextran) to synthetic biodegradable polymers (e.g., PLA) as well as reaction conditions and molecular weights, hydrogels can

be created with a wide range of swelling and biodegradable properties with desired drug release profiles. Another unique aspect of these newly invented biodegradable hydrogels is that they have controlled amounts of carboxylic acid pendant group. These groups could be used for sustained synthesis of acid rich hydrogels at the pH of the surrounding media as well as provide reactive sites for further attachment of anti-microbial and anti-viral agents.

The hydrogel, made of natural polysaccharide and synthetic biodegradable polymer, is hydrolytic, i.e. bioerodes slowly when in contact with water. The body easily absorbs the other ingredients.

2.2. Metal Salts

Metal ions such as calcium, sodium, magnesium, copper and iron act as spermicidal and/or spermiostatic agents. Daunter used copper EDTA – ascorbic acid fertilizing preventing agents that can be delivered via non-biodegradable, polyurethane or polyvinyl acetate discs to increase the viscocity of the cervical mucus and lower the pH of the vagina. Calcium and magnesium chloride demonstrated complete spermeostasis at concentration of 25Mm and 35Mm. Shoham et al. tested the use of copper wire on sperm penetration test into bovine mucus. Diveley tested the metal salts on 1,1,5,5-tetrasubstituted dithtobiurets as spermiostatic agent. Brode et al. used nonoxynol-9 as a spermiostatic delivered via hydrophobically modified polysaccarides as polymeric delivery system.

Currently, intravaginal barrier and intrauterine contraceptive devices, with or without hormones, are available to inhibit ovulation and to prevent sperm migration into the vagina leading to fertilization. A literature search for non-hormonal, non-toxic, and non-invasive contraceptive agents, anti-microbial agents and anti-viral agents revealed that metal ions and their derivatives, such as calcium chloride, sodium chloride, magnesium chloride, copper, and ferrous sulfate act as spermicidal and/or spermiostatic agents. Copper sulfate has been used in IUDs as a spermicidal agent. It is known that sulfl3ydryl groups are essential components of certain vital enzymes necessary for stability of the sperm. The copper-based agents are toxic due to their sulfhydryl binding properties and thus cause a direct deleterious effect on sperm. Copper also influences midcycle human cervical mucus by causing lysis of the mucus material, changing the physico-chemical properties of the mucus resulting in a decrease in sperm penetration.

Diveley tested metal salts of 1,1,5,5-tetrasubstituted dithiobiurets as spermiostatic agents. Light metals such as sodium and potassium, alkaline earth metals such as calcium and barium and heavy metals such as zinc, cadmium, tin, mercury, copper, nickel, chromium, iron, manganese, and cobalt, given orally as chelates, have shown to form dithiobiuret salts, which act as contraceptives and pregnancy terminators. Sawan, et al showed that insoluble, inorganic metallic salts and oxides of silver, magnesium, zinc, copper, cadmium or arsenic can

be used as anti-inflammatory agents. Brode, et al used benzylalkonium chloride, octoxynol-9, nonoxyl-9, ricinoleic acid, and phenol mercury acetates as spermicides delivered via hydrophobically modified polysaccharides as a polymeric delivery system to reduce the potential for infection and sexually transmitted diseases (STD). Cellulose-based vehicles consisting of hydroxyethyl cellulose and hydroxyethyl methylcellulose, or mixtures thereof, or optionally a cosmetic ingredient selected from the group consisting of water, ethyl alcohol, isopropyl alcohol, glycerin, glycerol, propylene glycol, and sorbitol, were also used as delivery systems. Typical forms of delivery systems used vaginally include creams, lotions, gels, foams, sponges, suppositories, and films. Daunter used Cu (II) ethylenediaminetetraacetic acid/ L-ascorbic acid, neuraminidase, and asialofetuin as fertility preventing agents that can be delivered via polyurethane or polyvinyl acetate discs. The first two agents act on the cervical mucus to change it from the open cellular structure found at midcycle of the menstrual period to the closed structure in order to form an impenetrable barrier for sperm. An ethylene vinyl acetate copolymer has also been used as a component of the matrix for the intravaginal device. Albumin increases the viscosity of the cervical mucus by diminishing the effect on ferning and spinnbarkeit. Albumin, dextran, and vinyl acetate were found to affect mucus spinnbarkeit due to the polymerization of the mucous glycoprotein, resulting in an increase in the viscosity of the cervical mucus. The spermicidal effect of

certain devices was also based on their ability to change the vaginal pH to become more acidic.

2.3. L-Ascorbic Acid

The secretory cells of the mucosa of the cervix produce a secretion called cervical mucus, a mixture of water, glycoprotein, serum-type proteins, lipids, enzymes, and inorganic salts. Females of reproductive age secrete 20-60 ml of cervical mucus per day. Cervical mucus is more receptive to sperm at or near the time of ovulation because it is less viscous and more alkaline with a pH of about 7.5-8.5. After the ovulation, the mucus becomes very thick and forms a cervical plug that is physically impenetrable to sperm. And then the cycle repeats, with the mucus becoming less viscous as ovulation approaches and thicker afterwards. At the commencement of the menstrual cycle, cervical mucus has a tight "honey-comb" cellular structure with a channel diameter of 2-6 mμ•m, which forms an impenetrable barrier to sperm. At midcycle, the channel diameter is 30-35 mμ•m in order to allow the sperm to pass. At the luteal phase, the cellular structure again contracts to 2-6 mμ•m and the mucus becomes more viscous.

Hence, if the viscosity of the cervical mucus was increased during the menstrual cycle, then sperm mobility would be impeded. L-ascorbic acid is an antioxidant, transferring electrons to change the conformation as well as reducing agent for disulfide (-S-S-) bonds of

mucopolysaccharides of glycoproteins of the cervical mucus, resulting in closed cellular structure of the mucus from the open cellular structure found at midcycle of the menstrual period and thus form an impenetrable barrier ior sperm. As pointed out by Jones and Mann ascorbate re-reduces Fe^{3+} to Fe^{2+}, thus acting catalytically. It transfers electrons to the mucopolysaccharides. The increased viscosity would inhibit sperm motility. The increase in the viscosity of the cervical mucus serves as the second line of resistance for the sperm to reach the ovum.

3. Material efficacy

Preliminary studies have been conducted to determine to efficacy of the material selected. The research included the following:

1. Determination of the efficacy of ferrous gluconate on sperm motility as a spermiostatic agent
2. Effect of L-ascorbic acid in increasing the viscosity of the cervical mucus.
3. Development of three generations of biodegradable matrices for controlled release of ferrous gluconate and L-ascorbic acid and to determine their effects on sperm motility and on the viscosity of the cervical mucus.

4. Effect of polyamino-polycarboxylic acid chains and acid-rich new hydrogels in order to obtain a pH of 5 of the hydrogel eluates.

5. Spermiostatic effect of a hydrogel containing ferrous gluconate and ascorbic acid on rabbit sperm. in in vitro and in rabbit model.in vivo.

3.1. Effect of metal salts and chemicals on sperm motility

Initially, the effects of various concentrations of magnesium chloride, calcium chloride, and ferrous sulfate (Figure 1) as well as of copper sulfate and ferrous gluconate (Figure 2) on the motility of human sperm were studied *in vitro*. Calcium chloride and magnesium chloride were spermiostatic at concentrations of 25 mM and 35 mM, respectively, whereas, ferrous sulfate completely arrested the motility of human sperm at a concentration of 10 mM. Copper sulfate and ferrous gluconate were spermiostatic at concentrations of 6.25 mM and 12.5 mM, respectively (Figure 2). 6.25 mM solutions of copper sulfate and 12.5 mM ferrous gluconate caused 93.3% and 97.4% immobilization of sperm respectively. A 37.5 mM solution of both reagents completely immobilized all the sperm. On the basis of the above observations and information collected which is existing in literature, the iron salt in the form of ferrous gluconate ($C_{12}H_{22}FeO_{14}.2H_2O$) was selected and further evaluated as the spermiostatic agent. Ferrous gluconate is used at nontoxic levels, (US Pharmacopia suggested toxic level LD=50 4.5g/Kg body weight) is

biocompatible and is used as a nutritional iron supplement. Iron promotes lipid peroxidatlon. Lipid peroxidatlon is a type of cellular damage involving the formation of free oxygen radicals, such as super-oxide anion, O^{2-}. The radicals are extremely unstable and unfavorable to the lipid bilayer of a cell, resulting in cell damage. The lipid peroxidation process is initiated in human spermatozoa through intracellular production of reactive oxygen species overwhelming the antioxidant defense system, superoxide dismutase (SOD), used by the cell. Human spermatozoa are enriched with unsaturated fatty acids, and fatty acids are particularly susceptible to lipid peroxidation. Sperm are thus predisposed to peroxidative damage. This reaction occurs when lipid peroxides in the bilayer of sperm tails are exposed to ferrous ion resulting in the propagation of lipid peroxidation, which leads to a continuous formation and decomposition of lipid peroxides. Eventually, this causes structural damage, a decline in metabolic activity, and spermiostatic effects in sperm cells. Ferrous gluconate targets the sperm tail and causes lipid peroxidation as shown below.

$O_2 + e^- \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow O_2^-$ (This is a superoxide dismutase radical)

-- SOD removes O_2^-

$2O_2^- + 2H+ \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow H_2O_2 + O_2$

(Hydrogen Peroxide)

O_2^- are formed intracellularly within the mitochondria. Some of the e^-, passing via the e^- transport chain, leak from the e^- carriers and react with O_2 reducing it to O_2^-

(Fe^{2+})

$O_2^- + H_2O_2 \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow \rightarrow\rightarrow \rightarrow OH^-$ (Hydroxyl radical)

RH $\rightarrow \rightarrow$ R $\rightarrow \rightarrow$ $(O_2) \rightarrow \rightarrow$ ROO $\rightarrow \rightarrow$ (anti-oxidation) $\rightarrow \rightarrow$ ROOH

Lipid Lipid (Liquid Peroxyl) (Liquid Peroxide Radical)

Cell Damage $\leftarrow \leftarrow \leftarrow \leftarrow \leftarrow$ Propogation of $\leftarrow \leftarrow \leftarrow \leftarrow$ Addition of Fe^{2+}

Figure 1: Effect of various concentrations of $MgCl_2$, $CaCl_2$, and $FeSO_4$ on sperm motility

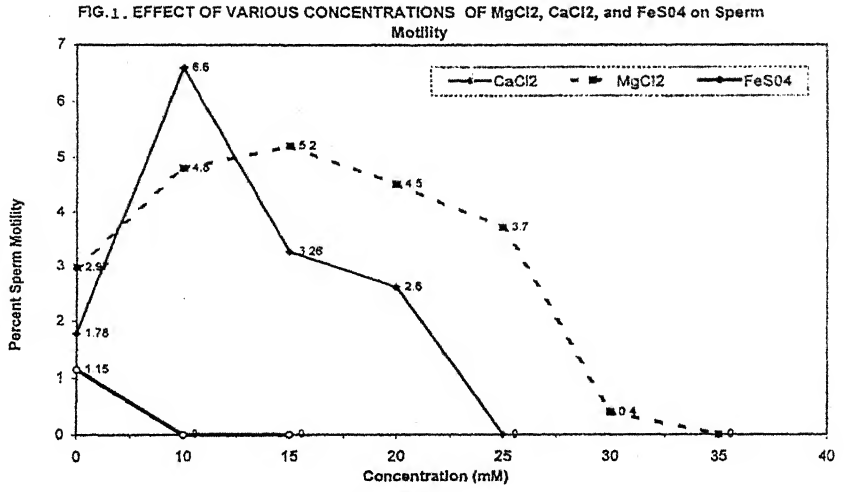
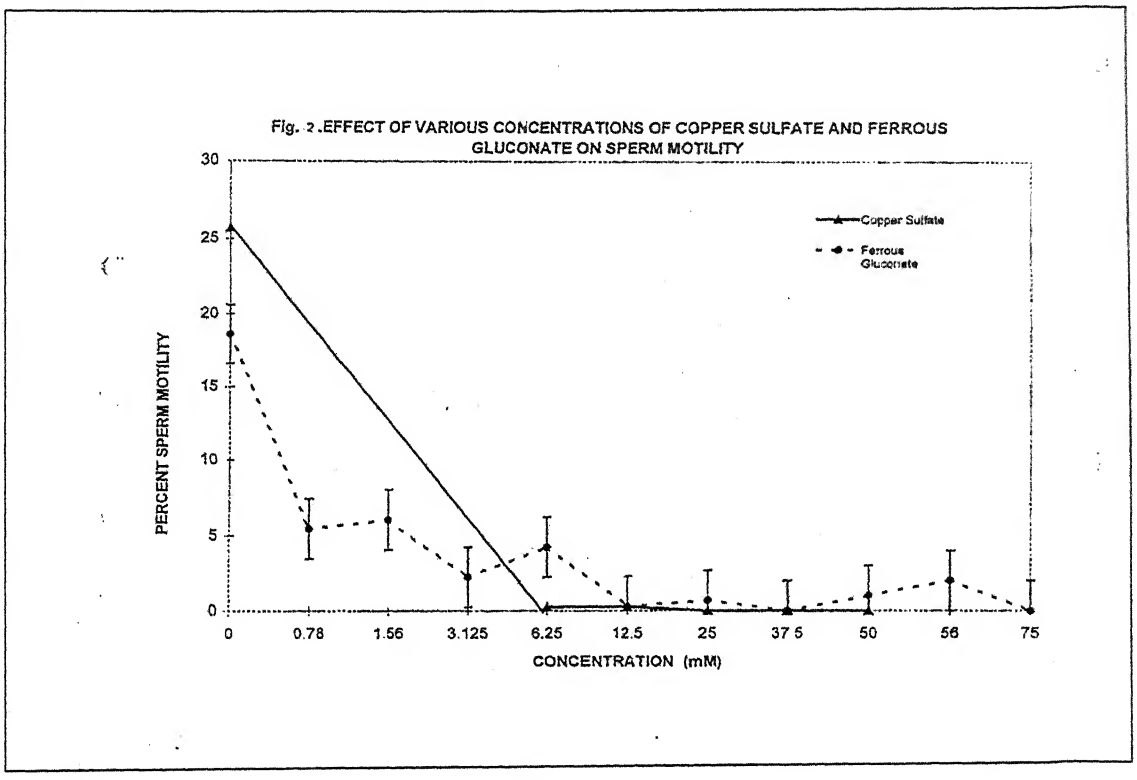


Figure 2: Effect of various concentrations of copper sulfate and ferrous gluconate on sperm motility



3.2. Effect of ascorbic acid on the viscosity of human cervical mucosa

L-ascorbic acid is water-soluble and thus can mix well with mucus as well as with semen. Table 1 shows the effect of various concentrations of L-ascorbic acid on the viscosity of cervical mucus from four women volunteers as determined by WHO criteria. It is evident from the cervical mucus score that overall viscosity of the mucus increased 1n direct relation to increasing concentrations of L-ascorbic acid from 0.31% to a range of 1-2.5%. Similarly, as shown in Table II, the daily

eluates for twelve consecutive days of hydrogel matrix DA containing ferrous gluconate and L-ascorbic acid also increased the viscosity of the cervical mucus equivalent to that of follicular and luteal phases of the cycle.

It may be pointed out that the concentrations of ferrous gluconate up to 25 nM and L-ascorbic acid up to 2.5% is significantly lower than the suggested toxic doses of iron, namely LD 50 = 4.5 gm/kg body weight and of ascorbic acid as LDSo = 1.2 gm/kg body weight respectively (US Pharmacopia).

Table 4: Cervical Mucus scores at various concentrations of ascorbic acid for four different samples

Parameters	% Ascorbic Acid Solution							
Sample 1	0%	0.31%	0.63%	1%	1.25%	2.50%	5%	10%
pH	(4)	(5)	(4)	(4)	(3)	(3)	(3)	(2)
Quantity (ml)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Viscocity	0	3	1	1	1	2	2	1
Ferning	3	2	0	2	0	0	0	1
Spinnbarkiet	1	0	1	1	1	1	1	1
Cellularity	3	2	0	2	0	0	0	1
Total*	7.3	7.3	2.3	6.3	2.3	3.3	3.3	4.3

Sample 2	0%	0.31%	0.63%	1%	1.25%	2.50%	5%	10%
pH	(8)	(2)	(2)	(3)	(3)	(3)	(3)	(2)
Quantity (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Viscocity	2	3	2	2	3	0	2	2
Ferning	3	0	0	0	0	0	0	1
Spinnbarkiet	2	1	1	1	1	1	0	1
Cellularity	2	2	0	0	0	1	0	1
Total*	9.2	6.2	3.2	3.2	4.2	2.2	2.2	5.2

Sample 3	0%	0.31%	0.63%	1%	1.25%	2.50%	5%	10%
pH	(4)	(4)	(4)	(4)	(4)	(2)	(2)	(2)
Quantity (ml)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Viscocity	0	2	1	2	1	1	2	1
Ferning	3	0	0	0	0	0	0	0
Spinnbarkiet	1	0	1	1	0	0	1	1
Cellularity	3	1	1	0	1	1	0	1
Total*	7	3.15	3.15	3.15	2.15	2.15	2.15	3.15

Sample 4	0%	0.31%	0.63%	1%	1.25%	2.50%	5%	10%
pH	(7)	(4)	(5)	(4)	(4)	(4)	(2)	(2)
Quantity (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Viscocity	3	2	3	2	3	2	2	0
Ferning	3	0	3	3	2	1	0	0
Spinnbarkiet	3	1	1	1	1	1	1	0
Cellularity	2	0	0	0	1	1	1	0
Total*	11.2	3.2	7.2	6.2	7.2	5.2	4.2	0.2

*pH not included in the total score

Table 5: Effect of daily eluates of the matrix on cervical mucus score

Parameter	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
pH	7	7	7	7	7	5	7	3	4	5	4	6
Viscocity	0	0	0	1	1	0	0	1	0	1	2	2
Ferning	1	0	0	2	2	0	0	0	0	0	0	0
Spinnbarkeit	0	0	1	1	0	0	1	1	0	1	1	1
Cellularity	2	2	2	2	2	0	0	0	0	0	0	0
Total*	3	2	3	6	5	0	1	2	0	2	3	3

*pH not included in the total score

Normal Average values (number of subjects) of the total cervical mucus score on various stages of menstrual cycle (Viscosity, Ferning, Spinnbarkeit and cellularity)

Follicular Phase3.6 (11)

Midcycle 13.1 (3)

Luteal Phase 4.4 (6)

4. Synthesis of Matrix

The 1st generation matrix consisted of an aliphatic polyester copolymer from PLA and poly (ε-Caprolactone) containing 24% ferrous gluconate by weight. Aliphatic polyesters have proven record in the biomedical field with predictable, biodegradation properties, have FDA approval, and are commercially available. The 1st generation Matrix was the simplest design to determine whether the concept of controlled release of spermiostatic agents from biodegradable matrix would be feasible.

The release data from the 1st generation matrix prompted us to employ a sandwich design concept, which was the 2nd generation matrix. The purpose of this sandwich configuration was to enhance the controlled release of the impregnated spermiostatic agent, particularly after the initial burst release. The center layer of the sandwich was a copolymer of PLA and Poly (ε-caprolactone) containing 38.7% of ferrous gluconate. The top and bottom layers were poly (ε-caprolactone) homopolymer (PCL) containing 21.5% ferrous gluconate by weight.

Since the release of ferrous gluconate from the 1st and 2nd generation biodegradable matrices showed a relatively short-lived 24-hour burst release profile and the matrices did not have sufficient ferrous gluconate remaining for a subsequent sustained release, a 3rd generation of biodegradable matrix was designed. This 3rd generation matrix employed a new biodegradable core and sheath design concept to provide a more

sustained and constant release over a longer period. This inner hydrogel core was covered by a biodegradable sheath. The objective of the hydrogel core was to provide sustained release of the contraceptive agents during the fertile stage of the menstrual cycle when the mucus is copious and women is in fertile period as well as to compensate for the declining concentration of the agents released from the sheath materials in the early stage. The intended purpose of the sheath materials was 3 fold. First, they would retard the onset of swelling of the hydrogecore and release rate during the early stage of application and hence preserve the impregnated contraceptive agents for later stage release. Second, the sheath materials could also restrict the initial burst release of drugs from the hydrogel core so that it would yield constant release of the incorporated agents from the hydrogel core. Third, the sheath material would be the source of ferrous gluconate for initial stage release. Since the sheath materials would be used to release ferrous gluconate in the initial stage and to delay and contain the release of this agent from the core, synthetic biodegradable biomaterials having good hydrophobicity and/or tight mesh structure were used. The core sheath design was expected and was indeed observed to provide sustained release of the incorporated spermiostatic agent over a desired period. By using a combination of a variety of coresheath design concepts, such as multicore-sheath design, a wide range of release profiles could be generated and tailored accordingly to specific clinical needs.

The biodegradable hydrogel cores used in the third generation were three-dimensional hydrogel networks consisting of dextran-PLA. The new technology combines the merits of natural biodegradable polymers like dextran with synthetic biodegradable polymers like PLA into a single entity (via chemical crosslinking) so that there would be no phase separation and thus better and more predictable release of the incorporated biochemical agents. By controlling the composition ratio of dextran (as hydrophilic component) to PLA (as hydrophobic component), a wide range of swelling properties (i.e., a wide range of drug release profiles), as well as differing degrees of hydrophilicity, and three dimensional porous network having pore sizes between 0.1 μ and 600 μ could be achieved. Both dextran and PLA are FDA-approved biomaterials and hence would be biocompatible and cost effective, and bring the clinical trials at a faster pace.

We had further developed 5 versions (A, B, C, D, DA) of the 3rd generation device and they were different in the number and type of sheath materials, concentration of the impregnated ferrous gluconate, and also the incorporation of L-ascorbic acid. The hydrogel core similar to the 3rd generation test samples was used for all 5 versions (A, B, C, D, and DA).

Their compositions are as given below.

1) Sample A

The core was made of dextran-AI hydrogel and had 2% ferrous gluconate by weight. The inner first sheath was made of the copolymer of ε-caprolactone and L-lactide containing ferrous gluconate (73.8% by weight of the polymer). The second sheath consisted of poly-ε-caprolactone and predetermined amounts of ferrous gluconate.

2) Sample B

It had the same hydrogel core as Sample A and had 2% ferrous gluconate by weight. The inner first layer contained poly-ε-caprolactone/poly-L-lactide copolymer and predetermined amounts of ferrous gluconate. The second layer was poly-ε-caprolactone homopolymer containing predetermined amounts of ferrous gluconate. The third layer was made up of poly-ε-caprolactone/poly-L-lactide/polyethylene glycol copolymer, without ferrous gluconate.

3) Sample C

The same hydrogel core as Sample A containing 2% ferrous gluconate by weight was used. The inner first sheath was poly-ε-caprolactone/poly-L-lactide copolymer containing predetermined amounts of ferrous gluconate. The 2ndinner sheath was of poly-ε-caprolactone-homopolymer containing predetermined amounts of ferrous gluconate.

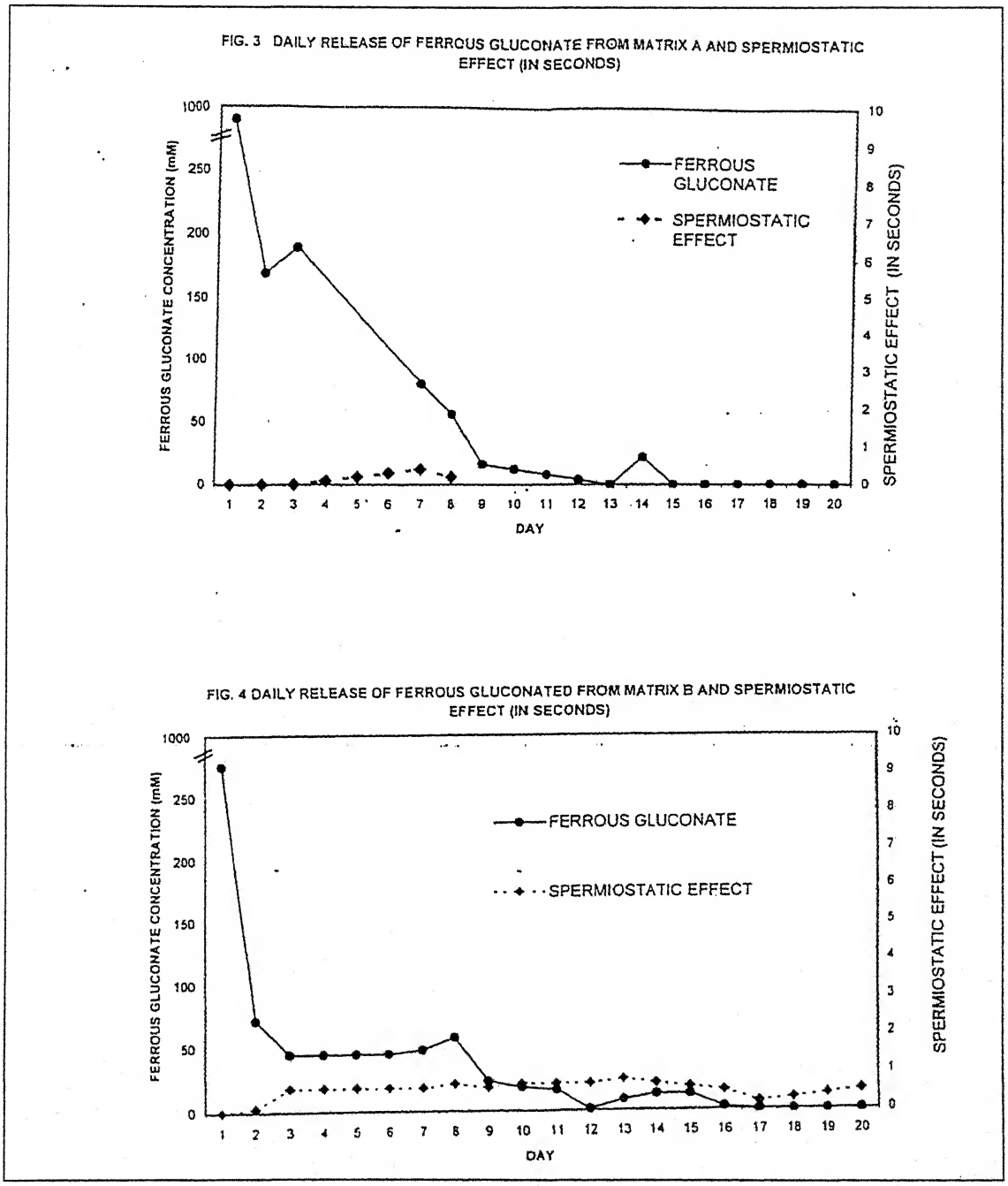
4) Sample D

The same hydrogel core as Sample A containing 2% ferrous gluconate by weight was used. This core material was coated by the following four layers of biodegradable polymers. The first layer was poly-D-L-lactide macromer impregnated with predetermined amounts of ferrous gluconate. The second layer was poly-ε-caprolactone/poly-L-lactide/polyethylene glycol copolymer containing predetermined amounts of ferrous gluconate. The third layer was poly-ε-caprolactone/poly-L-lactide copolymer impregnated with predetermined amounts of ferrous gluconate. The fourth layer also contained poly-ε-caprolactone/poly-L-lactide copolymer but was not impregnated with ferrous gluconate.

The ferrous gluconate release profiles from the first four 3rd generation samples are shown in Figures 3-6. Samples A (Figure 3) and B (Figure 4) showed efficacious spermiostatic activity for 8 days.

Figure 3: Daily release of ferrous gluconate from matrix A and spermiostatic effect (in seconds)

Figure 4: Daily release of ferrous gluconate from matrix B and spermiostatic effect (in seconds)



Thus, these two samples were the candidates for contraceptive devices of one-week duration; however, they were not sufficient for longer sustained release such as the 28-day period.

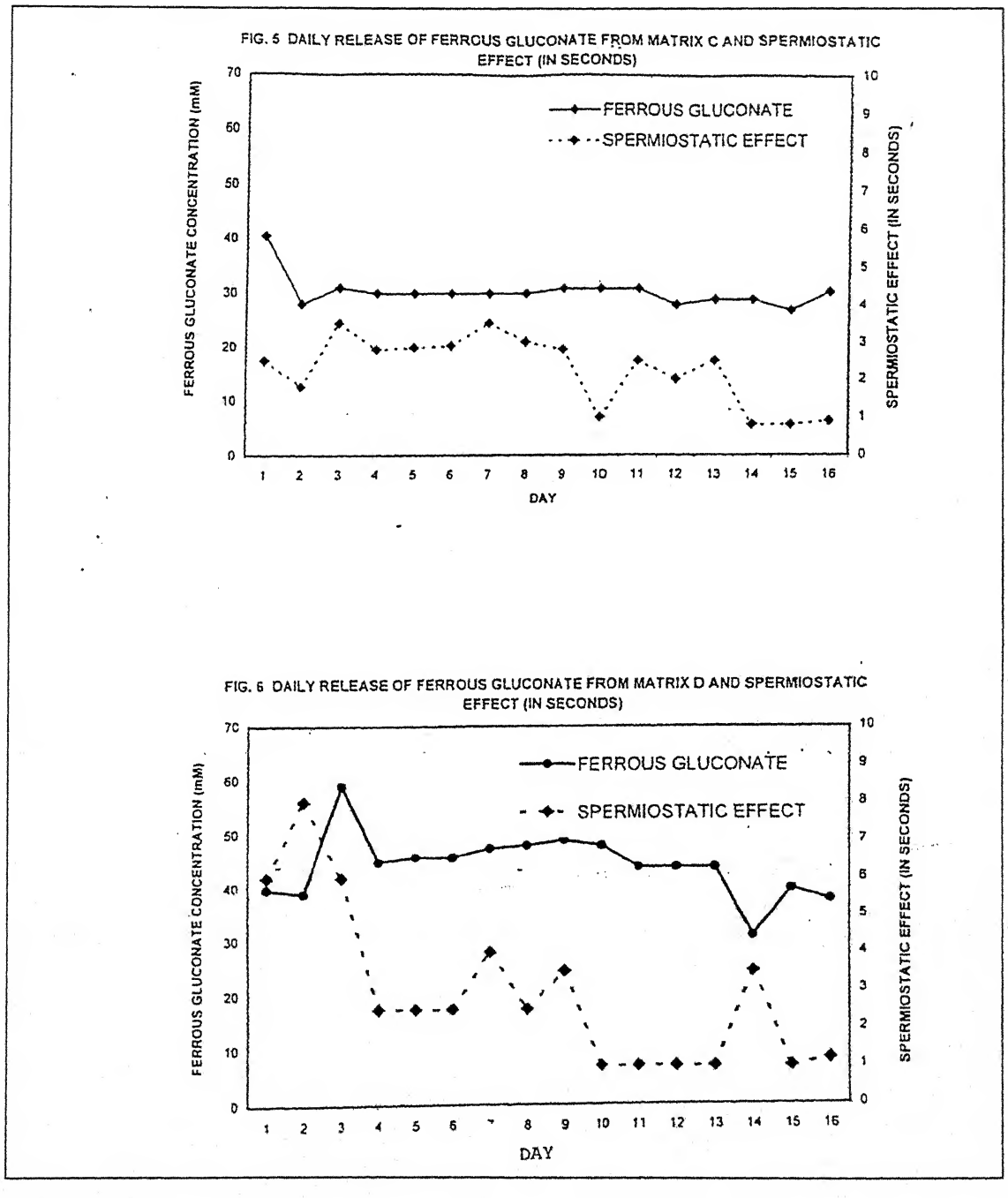
Samples C (Figure 5) and D (Figure 6) exhibited acceptable daily release rates of ferrous gluconate and with approximately, 33% and 42% biodegradability of the matrices, respectively, for a period of 16 days (Table 6).

Table 6: Change in the weight of the matrices C and D

Sample	Initial weight (gm)	Final Weight (gm)	Differences (gm)	Days
Sample C	0.5541 (gm)	0.375 (gm)	0.179 (gm)	16
Sample D	0.5406 (gm)	0.306 (gm)	0.234 (gm)	16

Figure 5: Daily release of ferrous gluconate from matrix C and spermiostatic effect (in seconds)

Figure 6: Daily release of ferrous gluconate from matrix D and spermiostatic effect (in seconds)



However, sample D showed the best sustained controlled release among all three generations of-matrices and appears to have the potential for delivering efficacious spermiostatic agents for longer periods. Hence, sample D appeared to be the best starting candidate to incorporate ferrous gluconate and ascorbic acid for the preliminary study.

5) Sample DA

Sample DA was co-loaded with both ferrous gluconate and ascorbic acid. The same hydrogel core as Sample D containing 2% ferrous gluconate by weight In addition, the hydrogel core consisted of predetermined amounts of L-ascorbic acid and, photoinitiator 2,2-dimethoxy-2phenyl acetophenone, and N,N'-dimethyl formamide. The inner first layer was composed of PDLLA macromer, ferrous gluconate, L-ascorbic acid, photoinitiator 2,2-dimethoxy-2-phenyl acetophenone, and N,N'-dimethyl formamide. The second inner sheath contained lactide/caprolactone/ethylene oxide copolymer and predetermined amounts of ferrous gluconate, L-ascorbic acid, and chloroform (6% by weight). The third layer was made up of lactide/caprolactone copolymer, ferrous gluconate, L-ascorbic acid, and chloroform, again 6% by weight. The daily eluates from the matrix DA were analyzed for ferrous gluconate and ascorbic acid as described earlier (Figure 7A and 7b). The spermiostatic effect and effect on the increase in the viscosity of the cervical mucus of the eluates are shown in Figure 8A and Table 5, respectively. The spermiostatic effect of the eluates was tested for 11

days and increase in the viscosity of the cervical mucus was tested for 12 days. As shown in Fig. 8a, the spermiostatic effect was achieved within 10 seconds and the pH of eluates was stabilized approximately at 5. The ongoing experiments have confirmed that a combination of iron and ascorbic acid has an efficacious effect as a spermiostatic agent and is effective in increasing the viscosity of the cervical mucus.

Figure 7: 7a-Daily release of ferrous gluconate from hydrogel matrix DA and 7b-Daily release of ascorbic acid from hydrogel matrix DA

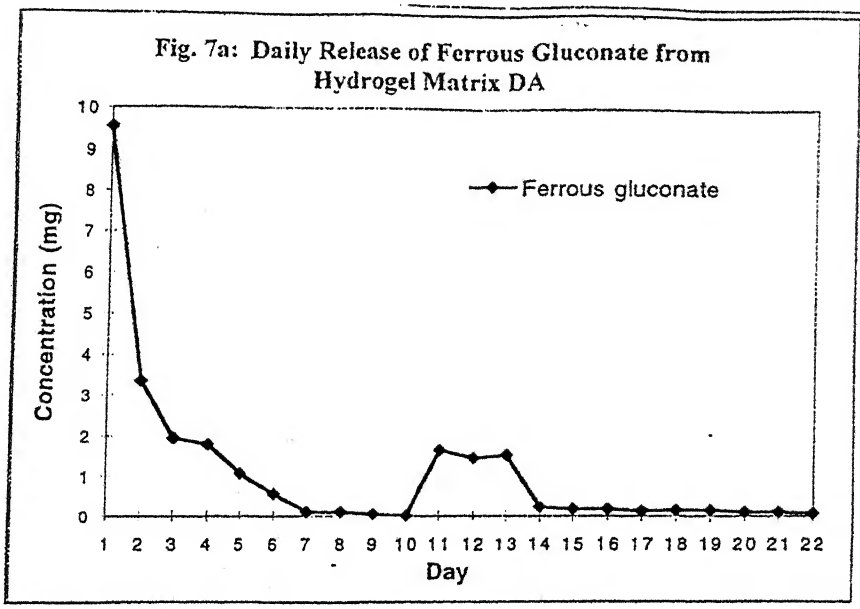


Fig. 7a: Daily Release of Ferrous Gluconate from Hydrogel Matrix DA

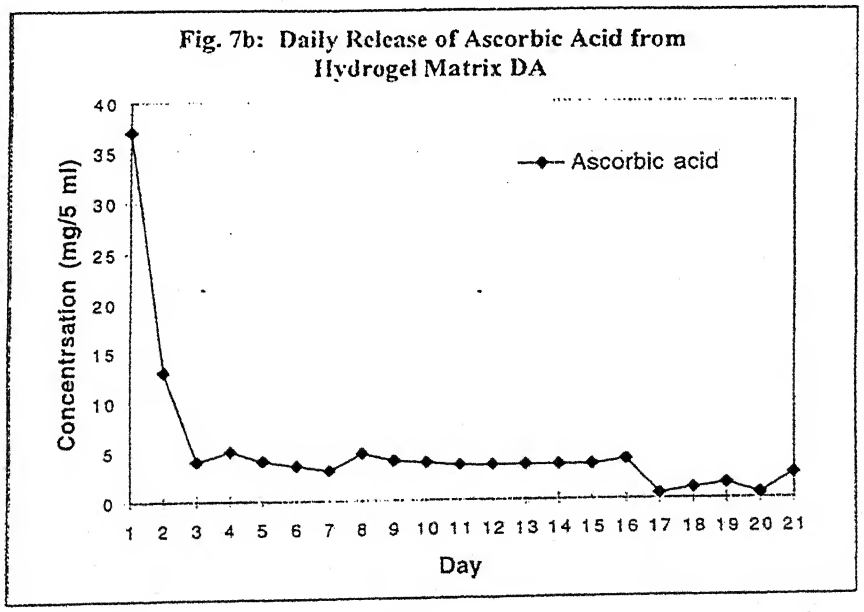
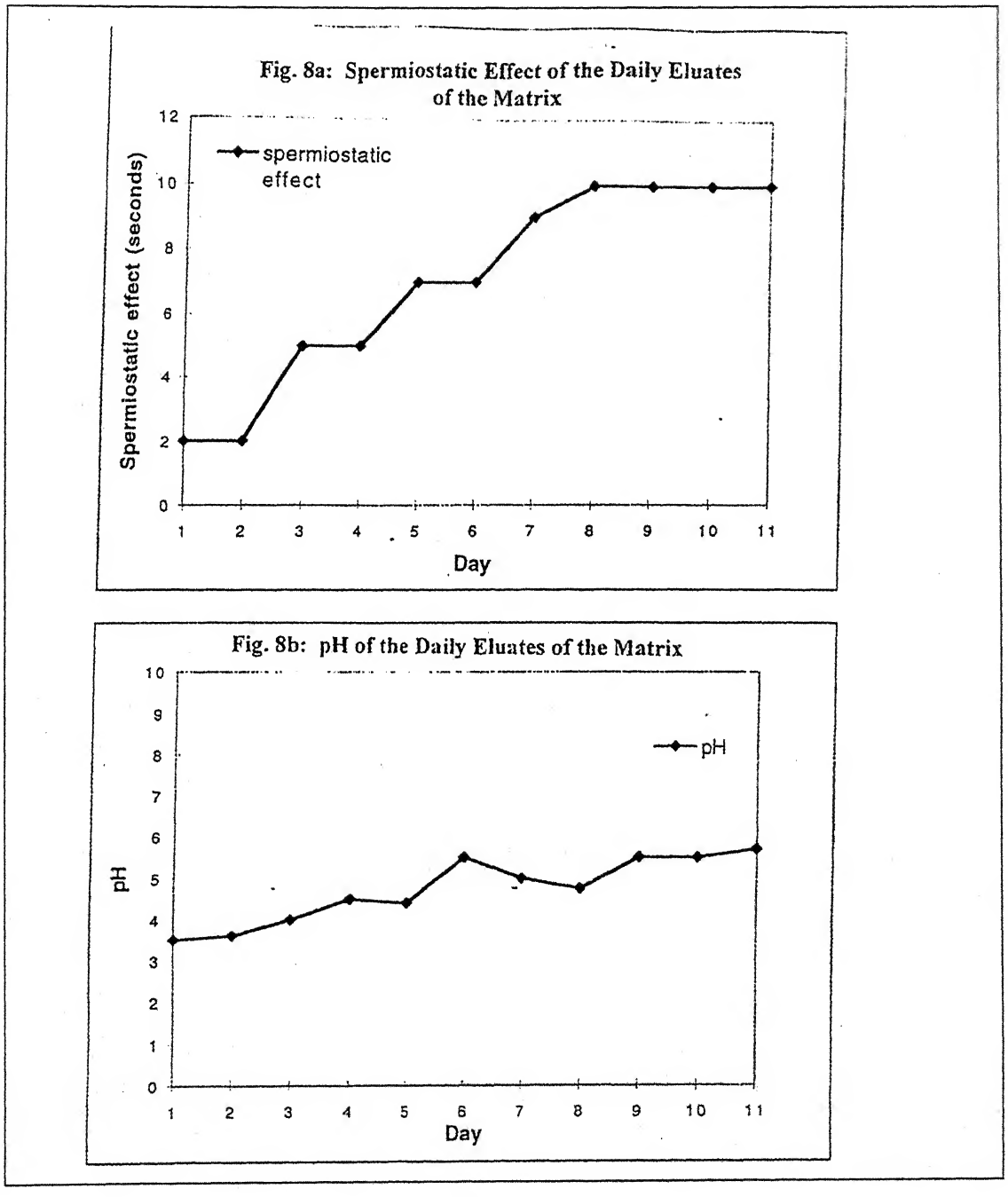


Fig. 7b: Daily Release of Ascorbic Acid from Hydrogel Matrix DA

Figure 8: 8a-Spermiostatic effect of the daily eluates of the matrix and 8b-pH of the daily eluates of the matrix



4.1. Composition of hydrogel matrix DA

Based on our preliminary studies, the basic design concept of the contraceptive delivery vehicle in this proposal will have a core-sheath structure. The hydrogel core will be coated by the four layers (the first requiring photo-cross-linking under LTV light at 365 nm for approximately, 1 day).

The hydrogel core:

Core Materials	Dextran-AL = 3.10 g
	Fe gluconate = 0.046 g
	L-Ascorbic acid= 0.0154 g

The 4 coated layers

First Layer	PDLLA macromer
	Fe gluconate = 0.023 g
	L-Ascorbic Acid = 0.0077 g
Second Layer	Poly (lactide & (-caprolactone copolymer)
	Fe gluconate = 0.0462 g
	L-Ascorbic Acid = 0.0154 g
Third Layer	Poly (lactide & (-caprolactone copolymer)
	Fe gluconate = 0.0616 g
	L-Ascorbic Acid = 0.030 g
Fourth Layer	Poly (lactide ε-caprolactone copolymer)

4.2. Acid-rich Hydrogels

Based on our preliminary studies acid-rich biomaterials were used to synthesize biodegradable hydrogels as an alternative to the use of polyamino-poly-carboxylic acid mixtures. The dextran/PLA hydrogel cores were synthesized according to previously published methods. The numbers of the free-COOH groups were modified by changing the reaction conditions in the synthesis of dextran-maleic acid and a co-poly (ester-amide), as an acid donor to the surrounding medium.

Furthermore, we tested to determine, that in addition to being the vehicle for controlled delivery of spermiostatic agents, whether our newly invented acid-rich biodegradable biomaterials could also serve as an acid donor to make the surrounding medium acidic for enhancing the spermiostatic activity. The numbers of the free -COOH groups could also be modified to provide the preferred acidic environment by changing the reaction conditions for augmenting the spermiostatic effect. Such an approach was an alternative to using the poly-amino-polycarboxylic acid mixture as described above. In this study, we immersed a fixed amount of dextran-maleic acid hydrogel and a co-poly (ester amide) separately in distilled water and the pH of the water was measured for an extended period. Table 7 summarizes our findings. These preliminary data illustrate that our newly invented biodegradable biomaterials could not only be used as the hydrogel core and/or sheath materials for this

proposal but could also have the advantage of providing an adequate acidic environment for impeding sperm motility. These newly invented biodegradable hydrogels used as the core materials have additional benefits. First, the hydrogel precursors (dextran, dextran-maleic acid, and PLA) have excellent solubility in various common organic solvents (dextran and dextran-maleic acid also dissolve in water). Such good solubility is vital for easy and cost effective means to formulate the contraceptive devices. Secondly, the availability of the built-in free carboxylic acid groups in the hydrogel macromolecules will have many useful functions. The free - COOH group would be able to not only enhance the solubility in a polar solvent but also provide the chemical reactive site in the hydrogel for subsequent chemical attachment of other anti-STD and anti HIV desirable agents in future.

Table 7: Change of pH of aqueous medium in the presences of acid-rich biodegradeable biomaterials.

Immersion Time (Days)	Dextran-Maleic Acid	Co-Poly (ester-amide)
	pH	pH
0	6.20	6.28
1	4.33	5.31
3	4.39	4.41
8	4.42	4.31
15	5.20 I	4.17

4.3. Preparation of the hydrogel core

The preparation of the dextran/PLA hydrogel cores was based on published reports and patent applications. In brief, the preparation of hydrogel cores involves two major steps. The first step is the incorporation of unsaturated groups into dextran and PLA, with degree of substitution (DS) used to indicate the level of such incorporation. For example, a higher DS indicates a higher level of unsaturated group incorporation. As we have described earlier, the DS has a profound impact on the rate and extent of diffusion of the incorporated spermiostatic agents out of the hydrogel cores. The purpose of the unsaturated groups was to provide photo-crossiinking capability between dextran and PLA. Dextran of molecular weight from 43,000 to 70,000 will be purchased from Sigma. PLA of molecular weights about 800 to 8,000 will be obtained from Boehringer-Ingelheim.

Two types of dextran derivatives and one type of PLA derivative was synthesized for this project. The two types of dextran derivatives synthesized were dextran-maleic acid and dextran-allyl isocyanate. In dextran-maleic acid, the unsaturated groups will be linked to dextran via ester linkage. In the case of dextran-allyl isocyanate, the linkages between the unsaturated groups and dextran were urethane bonds. Because of the differing sensitivities of the ester linkages (dextran-maleic acid) and urethane linkages (dextran-allyl isocyanate) toward hydrolytic

degradation, we expected different time-dependent swelling of the hydrogel cores for the different types of dextran derivatives used. This suggests that we could control the release rate and extent of the impregnated spermiostatic agents by controlling the type of dextran precursors. Dextran-maleic acid based hydrogels also have one unique advantage: the availability of controlled number of free --COOH group that can be used to provide acidity to impede sperm motility as well as sites for further chemical reactions to attach desirable biochemical agents.

The 2nd step for developing dextran-PLA hydrogel cores would be the synthesis of PLA diacrylate macromers (PLAM) that would have the two same unsaturated groups (i.e., acrylate) chemically introduced at the two chains ends of each PLA macromolecule. The last step of developing hydrogel cores from both dextran derivatives and PLAM precursors will be photo-crosslinking of these two precursors in the presence of very small amounts of photoinitiators. In this last step, fixed amounts (5-20% by weight) of spermiostatic agent, like ferrous gluconate were introduced into the precursor solution before crosslinking. Long wavelength UV lamp will be used for photo-crosslinking. The duration of UV exposure could be adjusted to control the level of crosslinking, and hence the swelling and drug release profiles. Although we did not find any adverse effect of UV exposure on the spermiostatic agents in our study, we plan to test the efficacy of the spermiostatic agents upon UV exposure in this

proposal. Optimum concentrations of various spermiostatic agents, as determined from Aims 1-3 for their efficacious release for a 28-day period, were incorporated into the newly synthesized biodegradable hydrogel cores.

Based on our preliminary data, the release profiles of spermiostatic agents from hydrogels depended on hydrogels' swelling properties, which, in turn, are controlled by several material parameters, such as initial molecular weights of dextran and PLA materials, dichroism spectra (DS) of dextran and PLAM, the composition ratio of dextran to PLAM, UV irradiation time, and photo-initiator concentration. In this study, we examine these material parameters to determine the optimum release profiles of spermiostatic agents over a period of 28 days.

4.4. Preparation of Sheath Materials

As described in the preliminary study section, the functions of sheath material hydrogel cores were to slow down the water penetration into hydrogel cores and to retard the onset of the burst release of the incorporated spermiostatic agents during the initial stage and hence to provide a constant and sustained release of the agents. Therefore, synthetic hydrophobic biodegradable polymers like aliphatic polyesters and their copolymers were good candidates because they are FDA approved, biocompatible, have a proven record in medicine, have a predictable biodegradation property, are hydrophobic, and are

commercially available. Some possibilities for biodegradable aliphatic polyester candidates as the sheath materials could also be PLA, poly-ε-caprolactone, and the copolymers of lactide and poly-ε-caprolactone.

4.5. Design of Biodegradable Core-Sheath Matrices

The hydrcgel cores and sheath materials already developed we reassembled in this phase to fabricate biodegradable core-sheath matrices for subsequent *in vitro* and *in vivo* testing of their efficacy and biocompatibility. Based on our preliminary data, we prepared a hydrogel cores with variety of combinations and the sheath materials. The goal was to produce the most sustained, consistent, and prolonged release of the spermiostatic agents that would have the maximumspermiostatic efficacy. Two examples of such core-sheath matrices are given below to illustrate the design principle.

a) Two-hydrogel core design: In this design, there were two hydrogel cores separated by several layers of biodegradable hydrophobic polymer sheath. The objective of the inner core was to facilitate the sustained release of the impregnated agents during the late stage of application. The outer core was used to improve the release of the spermiostatic agents in the mid menstrual cycle. The inner and outer cores could also made from either the same or different hydrogel precursors or from the same hydrogel precursors, but with different DS, or different tightness of the three-dimensional

network structure. A prolonged and more sustained release would require a tighter three dimensional network structure, with higher DS. The insulating materials that separated the two cores wreathe sheath materials described in 4B. These sheath materials had the spermiostatic agents impregnated at different concentrations. There were several options for the number of sheath layers and their thickness. We expected fewer layers and/or a thinner-sheath layer to accelerate the release of the incorporated spermiostatic agents.

b) Five-hydrogel core design: In this design, we divided the desirable release duration into finer periods, i.e. early, early-middle, middle, middle-late, and late stages. Such a finer division of the release periods would permit us to fine-tune the release profiles to permit even smoother and more sustained release. The innermost layer would be for the late stage release; the next innermost layer was for the middle-late stage and so on, with the outermost layer for the early stage. These hydrogel cores were separated by sheath materials in the same manner as the two-hydrogel core design.

The possible experimental parameters to control drug release profiles from our hydrogel coresheath design would the material parameters (e.g., type of materials, their molecular weight, and (DS) and design architecture (e.g., arrangement of core vs. sheath) of the carriers. The release data obtained from the proposed in vitro and in vivo tasks were

used to alter accordingly either the materials or/and architecture parameters. We tested these parameters for tailoring the drug release profiles to meet any specific needs. In order to contain the cost and duration of this proposal, we would mainly focus on the biomaterial parameters with a few variations in the architectural design parameters because we believe that the biomaterial parameters would have a larger impact on the results than the architectural design parameters.

4.6. Characterization of the Biodegradable Core-Sheath

The newly synthesized hydrogel precursors (i.e., dextran-maleic acid, dextran-ally isocyanate, and PDLAM), hydrogel cores, and the sheath materials were characterized by standard polymer characterizations like FTIR, NMR, elemental analysis, thermal and mechanical analyses, and surface morphology by scanning electron microscope. For the hydrogel cores, we would characterize additional features like swelling properties, pore size, surface area, and interior morphology. The swelling behavior was the most important factor to regulate all other essential properties of hydrogels, such as permeability to bioactive agents, biocompatibility, rate of biodegradation, and mechanical properties. Mechanical properties of hydrogels would affect their structural integrity and dimensional stability and would give information about the ability of the hydrogel to resist pressure. The pore size/volume, surface area, and cross-sectional interior morphology would help to qualitatively evaluate the suitability of

pore size and porosity of hydrogels for drug anchorage and release. Mercury intrusion porosimetery used to quantify the average pore size, distribution, and pore volume of the hydrogels. BET surface area analysis will be used to determine the surface area of the dimensional hydrogels.

The effect of immersion in buffer solutions at 37°C on the chemical, physical, swelling and morphological properties of the biodegradable core-sheath matrices was also studied. Two pH buffer media were chosen for this immersion study with pH's of 7.4 and 5. The duration of the immersions was 1, 7, 14, and 28 days. The resulting information from this immersion study was correlated to the release data of spermiostatic agents from the biodegradable core-sheath matrices. Such a correlation provided us with the required structure-property information to fine-tune both the biomaterial and architectural design parameters to achieve the most desirable release profiles of the incorporated spermiostatic agents for meeting specific clinical needs. A typical example of such a correlation would be how the changes in the composition ratios of the two hydrogel precursors (dextran derivatives and PDLAM) would affect the swelling property of the hydrogel and hence its release profiles. If the release profile of a particular biodegradable core-sheath matrix was found to be undesirable, we would examine its swelling property, which was controlled by the composition ratio as well as other material and architectural parameters. We could then subsequently alter these

parameters to achieve the desirable swelling property, which would then lead to the desired release profiles. Biodegradable matrices containing predetermined concentrations of spermiostatic agents as well as, agents which will increase the viscosity of the cervical mucus, and those that will create an acidic environment were evaluated for daily and cumulative release rates in vitro as described below.

5. Calculation of release rates

1) Determination of Daily and Cumulative In Vitro Release Rates of Ferrous Gluconate from Eluates of the Biodegradable Core-Sheath Matrices:

   a) Cut pieces of the matrix and record the weight. Place them in separate petri dishes and label.

   b) To the pieces of matrices containing ferrous gluconate (dextran and polylactide). Add 5 ml of phosphate buffered saline (PBS) or water to the petri dishes and seal with parafilm. Incubate at 37°C overnight in a shaker.

   c) Each day collect the eluate and measure the volume. Transfer the matrix to a new petri plate in 5 ml of fresh PBS or water. Follow Step (b).

   d) Continue the above procedure daily for 20 days. Dry the residual matrix and record the final weight.

e) Determine the amount of ferrous gluconate in each daily collection of the eluate, as follows.

f) Test an aliquot from each eluate for the determination of spermiostatic activity (by semen analysis, as described earlier).

g) Record the pH of the eluates.

* Water will be used as the solvent when testing the ampholines or acid-rich matrix for the maintenance of acidic pH.

2) Determination of Cumulative Release Rates of Iron from Eluates of Hydrogel Matrix

a) Cut out pieces of the matrix to fit in the petri dish and record the weight.

b) Add 5 ml of PBS or water.

c) Each day collect 1 ml eluate from the petri dish and replenish with fresh PBS or water to make up the final volume to 5 ml.

d) In the aliquots collected, determine ferrous gluconate content and test an aliquot for spermiostatic activity.

3) Determination of Ferrous Gluconate by 1,10-Phenanthroline Method:

a) Principle: Phenanthroline forms an orange-red colored complex with the ferrous ion, which can be measured in a spectrophotometer. The color is stable for days. Hydroquinone reduces any ferric iron that may have been formed due to oxidation

of ferrous exposed to the environment. All solutions used should have a pH of 3.5.

b) Reagents:

- Prepare stock solution of 10x phosphate buffered saline (PBS).
- Prepare 1% solution of hydroquinone.
- Prepare 0.5% solution of 1,10-Phenanthroline. (Keep in dark and discard 1f any color develops).
- Sodium acetate-acetic acid buffer solution of pH 4 is prepared by dissolving 27 grams of anhydrous sodium acetate in 50 ml of distilled water. Add 24 ml of acetic acid and dilute to 100 ml.
- Standard (50.0 mM) ferrous gluconate solution is prepared in PBS by dissolving 0.224 g in 10 ml PBS.
- Bromophenol blue dye indicator. (pH range 3.0-4.6)

c) Procedure: Take 600 ml from each of the unknown solution or samples (containing approximately 0.01-0.02 mg ferrous gluconate) and transfer to 5 ml test tubes. For the Standard, prepare 0.5 mM ferrous gluconate in PBS in serial dilutions. Take 600 и1 from each of the known serial dilution of the standard solution and transfer to 5 test tubes. Using micropipet, add one drop of bromophenol blue followed by one drop of sodium acetate to the test tubes. To each tube, of the standard solution and each of the unknown solution, add 1 of 1% hydroquinone, followed by 1

ml of 0.5% 1,10-Phenanthroline. Vortex each tube gently. Allow mixture to stand for one hour or longer at room temperature. Read transmitkance at 408 nm using a spectrophotometer. Plot values derived from standard solutions of ferrous gluconate. From the absorbance curve, the concentration of ferrous gluconate in the eluates can be determined.

4) Determination of L-Ascorbic Acid:

In this procedure, ascorbic acid is oxidized to dehydroascorbic acid and the latter is coupled with 2,4-dinitrophenylhydrazine. The coupling reaction forms the 2,4-dinitrophenylosazone of dehydroascorbic acid, a light-brown crystalline compound. When treated with 85% $H2sO4$, the osazone is rearranged to form a reddish colored compound, which absorbs maximally at 500 to 550 mμ is a highly stable product under the conditions used and is well suited to colorimetric measurement.

a) Reagents

- Trichloroacetic acid solutions, 6% and 4%.
- 2,4-Dinitrophenylhydrazine reagent. Dissolve 2.0 gm of 2,4-dinitrophenylhydrazine in 100 ml 9 N H_2SO_4 (1 part of concentrated $H2sO4$ plus 3 parts of water). Add 4 gm of reagent grade thiourea, shake occasionally until dissolved, and filter. Keep in a refrigerator.

- Ascorbic Acid Standard Solutions:
  - Stock solution. Dissolve 50.0 mg of ascorbic acid of the highest purity in 100 ml of 0.5% oxalic acid. Keep in the refrigerator.
  - Standard solution of dehydroascorbic acid. Place 2 ml of the ascorbic acid stock solution in a 100-ml volumetric flask and make up to volume with 4% trichloroacetic acid solution. This solution is oxidized by adding 1 teaspoonful or (1 g.) of acid-washed Norite per 50 ml, shaking thoroughly, and filtering through Whahnan No. 42 filter paper. One ml of this solution contains 10 ~ig of dehydroascorbic acid. Keep in the refrigerator.
  - Preparation of Solution Filtrate: To one volume of solution, add 19 volumes of 4.0% trichloroacetic acid. This dilution will serve for a range of 1 to 300 mg of ascorbic acid per liter of solution.
  - Procedure: Place 4 ml of Norite filtrate of unknowns in each of two matched photoelectric colorimeter tubes. Place in another matched colorimeter tube of the dehydroascorbic acid standard solution (10 4µg per ml). To the standard tube and the tube containing Norite filtrate, add 1.0 ml of 2,4 dinitrophenylhydrazine reagent. The other tube containing

Norite filtrate is used as a control, no reagent being added to the tube at this time. Place the three tubes in a constant temperature water bath at 37°C. Keep the tubes immersed in the bath for exactly 3 hours. Remove and place them in a beaker of ice water containing generous quantities of ice. To each of the three tubes, while in the ice water bath, add slowly 5.0 ml of 85% H_2SO_4. Finally, to the control tubes, add 1 ml 2,4-dinitrophenylhydrazine reagent. The tubes are shaken under the ice water to achieve complete mixing and are then placed in a rack. After 30 minutes wipe the tubes dry and clean and record the absorption in a colorimeter using a 540 mμ filter. To take the reading, use the control tube to set the colorimeter at 100 % transmittance or zero absorbance.

5) Determination of Spermiostatic Effect by Semen Analysis

To test in vitro the efficacy of spermiostatic agents in terms of sperm survival and the time required to render human sperm completely immobile, routine semen analysis will be performed as recommended by WHO, with minor modifications. Semen samples with higher sperm counts will be appropriately diluted with phosphate buffered saline (PBS). Using a Neubauer counting chamber, a baseline count will be made by counting the non-motile and motile sperm. Thereafter, an aliquot of the semen sample will be placed on a

microtiter plate well and mixed with the known amount and concentrations of the agents being tested for spermiostatic effect. Four separate readings will be taken for each concentration of the agent used and the average counts will be calculated. The time required for the sperm to be completely immobilized will also be noted. The results will be calculated as the percentage of the total, untreated motile and non-motile sperm, and will be recorded. The optimum quantity of the spermiostatic agents to achieve maximum spermiostatic effect will be determined.

6) Determination of the effect, in increase the viscosity of the cervical mucus in vitro

   a) Collection of Cervical Mucus: Cervical mucus is produced by special cells in the walls of the uterine cervix as women approach their ovulation. Ovarian hormones regulate the secretion of cervical mucus; estrogen stimulates production of copious amounts of watery mucus and progesterone inhibits the secretory activity of the epithelial cells. The cervix is exposed with a speculum and the external os is gently wiped with a cotton swab to remove the external pool of vaginal contaminants. Cervical mucus is aspirated with a needle-less tuberculin syringe. The pH of the collected cervical mucus will be determined with pH paper (range 4.5 and 6.4-8.0). The optimum pH value for sperm migration and survival in the cervical mucus is between 7.0 and 8.5. Acidic mucus

immobilizes spermatozoa. Mucus is preserved either in the original tuberculin syringe and covered with parafilm to avoid dehydration. The samples are preserved in a refrigerator at 4°C for a period not exceeding 5 days. Usually the mucus specimens will be utilized within 2 days of collection. Various dilutions of L-ascorbic acid will be mixed with an appropriate aliquot of mucus and incubated for 30 minutes at 37°C in an incubator and the cervical mucus consistency will be determined. Cervical mucus consistency is scored as recommended by WHO as follows. Various parameters of cervical mucous consistency of untreated and treated mucus will be compared to determine the optimum amount of ascorbic acid needed to achieve desired viscosity of the mucus.

b) Viscocity

Score

0 = thick, highly viscous, premenstrual mucus

1 = mucus of intermediate viscocity

2+ = mildly viscous mucus

3+ = watery, minimally viscous, mid-cycle (pre-ovulatory mucus)

c) Spinnbarkeit: The cervical mucus placed on a microscopic slide is touched witha cover slip, or a second slide held crosswise, which is

lifted carefully. The length of the cervical mucus thread stretching in-between is estimated in centimeters and is scored as follows:

Score	Length (in cm)
0	= less than 1 cm
1	= 1 – 4 cm
2	= 5 – 8 cm
3	= 9 cm or more

d) Fernine: Ferning is due to decreased levels of salt and water interacting with glycoprotein on the mucus. Ferning is increased in capacity as ovulation approaches. Ferning is scored as follows:

Score	Description of ferning
0	= no crystallization
1	= atypical fern formation
2	= primary and secondary stems, ferning
3	= tertiary and quaternary stems, ferning

6. Preliminary in vitro studies on rabbit sperm.

At 25 mM concentration of ferrous gluconate, all the rabbit sperm were immobilized within 60 seconds. Spermiostatic effect of the eluates of the hydrogel DA impregnated with ferrous gluconate and ascorbic acid on

rabbit sperm was studied. Due to the necessity of using rabbit sperm on the same day initially daily eluates from five consecutive days were analyzed. Rabbit semen was diluted three-told with phosphate buffered saline to achieve counting efficiency of approximately 50 million/ml. Twenty µl of eluate was mixed with 20 µl of the diluted semen. In eluates with up to 1:3 dilution on Day 1 and Day 3, a11 sperm were immobilized instantaneously with shaking movement. Day 4 eluates caused immobilization with shaking or shivering, but no movement. Day 5 eluates showed slower immobilization and increased number (up to 10%) of shaking or shivering sperm. We now propose to proceed the in vivo experiment as described earlier.

7. Preliminary In Vivo Studies in Estrus Female Rabbit:

Female rabbit in estrus was selected using teaser males. The hydrogel was instilled into the anterior vagina by the aid of a wide insemination pipette at 9AM. The female was mated approximately 6 hours later to a male of known fertility. Post-coital flush was examined under a phase contrast microscope over a heated stage. All the sperm were found to be immobile or dead. Addition of human tubal fluid (Irvine Scientific, Irvine CA) did not revive the sperm. The experiment was repeated 72 hours later with similar results. An examination of external genitalia appeared normal without any adverse reaction.

8. Synthesis of BioRing

Having tested several generations of hydrogel matrices and selecting the optimum concentration of ferrous gluconate and L-ascorbic acid, Hydrogel DA is used to design the intravaginal device – BioRing.

8.1. Synthesis of Hydrogel DA for BioRing

The core and sheath matrices were characterized by Fourier transform infrared spectroscopy, nuclear magnetic resonance spectroscopy, elemental analysis, thermal and mechanical analysis, and surface morphology by the scanning electron microscope. Dextran of molecular weight range of 43,000-70,000 was purchased from Sigma Chemical Company (St. Louis, MO); PLA of molecular weight range of 800-8,000 was obtained from Boeringer-Ingelheim (Ingelheim, Germany). Chemical synthesis of the dextran derivative (Dextran-AI) was performed according to published procedures. The first step in the synthesis of the hydrogel core involved the incorporation of unsaturated functional groups into dextran with a degree of substitution consistent with the desired release rate of the spermiostatic agents. The unsaturated groups grafted onto the dextran backbone were then photocross-linked under UV-light at 365nm for 24 hours to form a dextran-based hydrogel network. The second step was the synthesis of sheath materials from three different types of synthetic biodegradable polymers. The first sheath layer was made from biodegradable poly (D,L) lactide diacrylate macromer

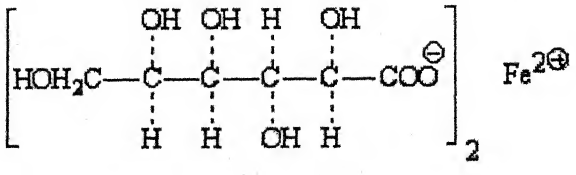
(PDLLAM) which had two unsaturated acrylate groups chemically introduced at the two chain ends of each PDLLA macromolecule. Fixed amounts of ferrous gluconate, ascorbic acid (range 5-20% w/w) and 5% concentration of Ampholines were introduced into the precursor solution. The last step was the photocross-linking of dextran derivatives and PDLLAM precursors by the addition of photo-initiators.

The core consists of dextran incorporated with predetermined quantity of contraceptive agents. Specified amounts of ferrous gluconate, ascorbic acid, and 5% of ampholines were introduced into the core and each layer except the outermost 4th layer. The fourth layer does not contain any contraceptive agents but helps to minimize initial burst effect. Finally, the hydrogel is photo-crosslinked under UV light at 365 nm for 24 hours to form a dextran based hydrogel.

8.2. Contraceptive agents incorporated in the hydrogel

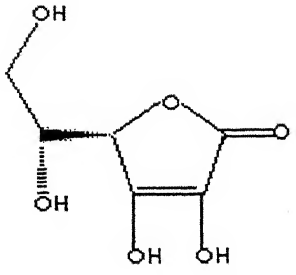
1. Ferrous Gluconate: Causes oxidative damage to the lipid bilayer of sperm tail by promoting lipid peroxidation leading to spermeostasis.

   Figure 9: Structure of ferrous gluconate



2. L-Ascorbic Acid: Acts as a reducing agent for disulfide (-S-S-) bonds of mucopolysacchrides of the cervical mucous resulting in confirmational changes from the open cellular structure found in midcycle to close cellular structure resulting in viscous cervical mucous that acts as a barrier to inhibit sperm penetrations.

Figure 10: Structure of L-Ascorbic acid

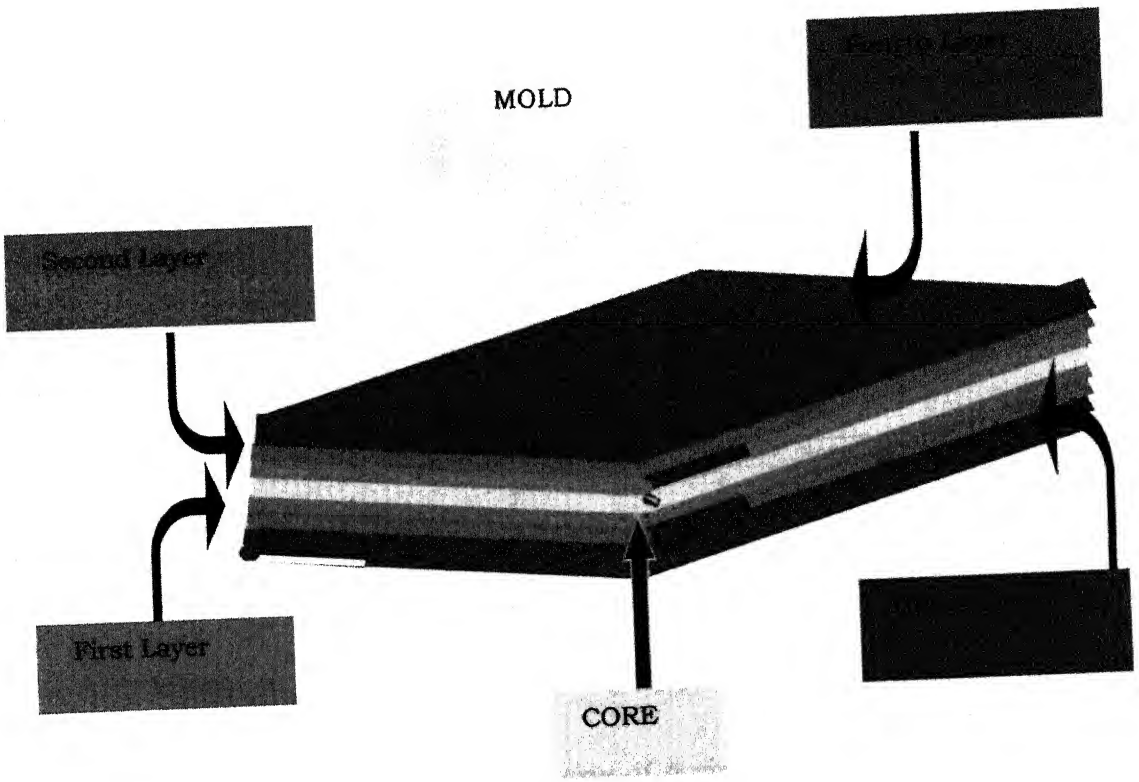


3. Ampholines: Polyamino -Polycarboxylic Acids

- Lowers the pH of semen (7.2 – 8.0) to 4.5 which is hostile to sperm
- Sustains the normal vaginal pH at 4.5

8.3. Formation of Hydrogel into a shape of a Ring

Figure 11: The BioRing



This delivery system is made up of a biodegradable hydrogel. The hydrogel is vastly used to make medical implants and wound closure devices, but has never previously been used as drug delivery system.

8.4. Composition of Hydrogel DA for BioRing

Table 8: Hydrogel composition

Hydrogel design	Matrix composition	Ferrous gluconate	Ascorbic acid	Ampholines
First layer	[illegible]	[illegible]	[illegible]	[illegible]
Second layer	Copolymer of polylactide, ε-caprolactone, ethylene oxide 0.5546 g	46.2 mg	15.4 mg	5 mg
Third layer	Copolymer of polylactide; ε-caprolactone 0.832 g	61.6 mg	30 mg	5 mg
Fourth layer	Copolymer of polylactide; ε-caprolactone 1.06 g	None	None	None

8.5. Estimation of release rates and efficacy

A portion of the hydrogel, in the shape of a ring, weighing approximately 2.5 g, was placed in a petri dish containing 10 mL of saline. The petri dish was closed airtight and placed on a shaker at a low speed in a 37°C incubator. The eluates from the gel were removed from the petri dish every 24 hours for 16 days. Each petri dish was replenished with 10 mL of saline volume. The eluates were stored in the refrigerator at 4°C and tested for pH as well as analyzed for the concentrations of ferrous gluconate and L-ascorbic acid as described below. The eluates were tested for spermiostatic activity and their effect on the viscosity of the cervical mucus. At the conclusion of the study, the remaining hydrogel

was removed from the petri dish and placed on filter paper to absorb adhering liquid and dry to a constant weight.

8.5.1. Determination of ferrous gluconate

For the standards, doubling dilutions of ferrous gluconate from 37 mM to 9.25 mM as well as from 9.25 mM to 0.58 mM were made in distilled water. Aliquots of 45 µL of each dilution of ferrous gluconate were pipetted in duplicates to the wells of a microtiter plate. Forty-five µL aliquots of daily eluate samples from the hydrogels were also pipetted as duplicates into separate wells of the same microtiter plate. The blank well contained only 200 µL of distilled water. Seventy-five µL of hydroquinone concentration and 75 µL of phenanthroline was added to all wells except the blanks. Finally, the total volume in each well was brought up to 200 µL by addition of the sodium acetate buffer. The micro plate was placed in the Jitterbug (Boekel Scientific, Feasterville, Pennsylvania) microplate incubator/shaker for 15 min to ensure thorough mixing of the reagents and for the development of the color. The plate was left to stand for one hour before the absorbency was recorded in a Multiskan Ascent (Titertek Instruments, Inc., Huntsville, AL) photometric microplate reader at 405 nm.

8.5.2. L-ascorbic acid release rates

Doubling solutions of L-ascorbic acid in concentrations of 56 μM, 28 μM, 14 μM, 7 μM, 3.5 μM, 1.75 μM as well as 448 μM, 298.67 μM, 224 μM, 149.63 μM, 112 μM were prepared in 4% trichloroacetic acid (TCA). Eighty μL of each dilution of ascorbic acid was pipetted in duplicates to the wells of a microtiter plate. Similarly, 80 μL of each eluate sample was pipetted in duplicates. There was one blank well that contained only 200 μL of 4% TCA. With the exception of the TCA blank, 20 μL of 2,4 dinitrophenylhydrazine was added to all the wells. The microplate was placed in the Jitterbug microplate incubator/shaker at a constant temperature of 37°C for three hours. At the end of the incubation period, 100 μL of 85% H2SO4 was added to all of the wells, except the blank, thus bringing the total volume in each well up to 200 μL. The microplate was placed on the Jitterbug shaker at medium speed for 30 min to ensure complete mixing of all the reagents. The absorbency in each well of the microplate was determined at 450 nm in an automatic Multiskan Ascent photometric microplate reader.

8.5.3. Determination of spermiostatic effect

A Diaphot-TMD (Nikon Inc., Garden City, NY) inverted, light microscope, connected to a COHU (Cohu, Inc., San Diego, CA) high performance, charge-coupled device camera was used to observe the sperm at a 100-fold magnification. In accordance with the World Health Organization

sperm count guidelines, a Neubauer counting chamber was used to evaluate semen samples. Using a stopwatch, the time was monitored when all sperm in the semen sample were immobile. The spermiostatic effect of magnesium chloride, calcium chloride, copper sulfate, ferrous sulfate and ferrous gluconate was tested by mixing 100 µL of various known concentrations of these different salt solutions in Tris-buffer with 100 µL of a thoroughly mixed semen sample. The time required for all the sperm to be immobilized was recorded for each concentration of each salt. The spermiostatic time values of the daily eluates of the hydrogels were determined in a similar manner. A total of 13 semen samples were examined.

8.5.4. Determination of cervical mucus viscosity

The effects of various concentrations of L-ascorbic acid on the cervical mucus from four female volunteers were observed. Cervical mucus specimens were collected and stored in sealed capillary tubes at 4° C. The specimens were analyzed according to World Health Organization guidelines within 24 hours after collection. Measurements were taken to obtain the mucus score, pH, viscosity, ferning and spinnbarkeit in known increasing concentrations of L-ascorbic acid. Similar testing was done by thoroughly mixing 40 µL of cervical mucus with 40 µL of daily eluates from the hydrogel.

8.5.5. Sperm penetration test

A sperm-cervical mucus test was conducted according to World Health Organization guidelines. The additive effects of the ferrous gluconate, ascorbic acid and pH were shown to create a reliable contraceptive. Forty μL of cervical mucus was thoroughly mixed with 40 μL of daily eluate samples from days 1 through 16. The mixture was placed on a glass slide and covered with a cover slip. Each semen sample was diluted with PBS such that the sperm count was approximately 50 x 10^6 per mL, thereby increasing counting efficiency. Ten μL of the diluted semen sample were pipetted at each corner of the cover slip, and the slide was placed in an incubator at 37° C. After 30 min, the slide was examined at a 20-fold magnification with a Nikon Diaphot-TMD inverted, light microscope.

8.5.6. In Vitro Effects on Lactobacillus

1) Vaginal swabs from patients having normal vaginal flora were placed directly into sterile culture tubes containing eluates of the hydrogel.
2) Culture tubes were incubated at 35°C for 24 hours to allow vaginal flora to possibly react with the eluates10 μL of each sample was plated on three different types of petri dishes containing Sheep blood agar, PEA agar and Conci agar and incubated for 24, 48, or 96 hours

4) Effect of the eluates on the vaginal flora was determined by examining each plate and comparing to the saline Control

8.5.7. In Vivo Studies in Rabbit

1) Semen was collected from a male rabbit of known fertility with the aid of an artificial vagina
2) The hydrogel was instilled into the anterior vagina of three female rabbits with a blunt, fire-polished pipette bent at a 50° angle to accommodate the vaginal contour
3) The female rabbits were inseminated 4 hours later
4) Post-insemination vaginal flushes were examined for sperm motility and examination of rabbit vagina was done to evaluate the local reaction.

Chapter 3: Results and Discussions

1. Results

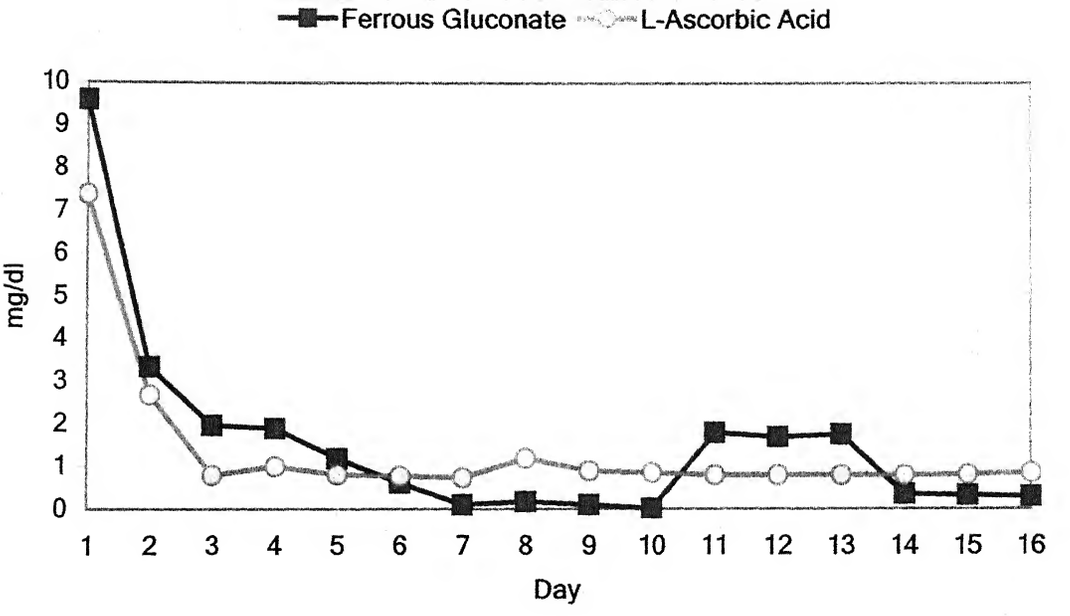
1.1. Efficacy of BioRing

The efficacy of hydrogel based contraceptive delivery device was established by utilizing in vitro and in vivo tests in the rabbit.

1.1.1. Daily release of ferrous gluconate and ascorbic acid

The daily release rate of ferrous gluconate and L-ascorbic acid for a 16-day period is shown in Figure 12. Initial burst effect is observed for the first 2 days. In case of L-ascorbic acid, the level drops of on Day 3 and more or less remains constant upto Day 16, whereas for ferrous gluconate, there are some variability observed. On Day 3, ferrous gluconate comes down and remains low up until Day 9, where on Day 10-14 it peaks. Theoretically, this is the mid-cycle ovulatory phase of a women's menstrual cycle. However, this window can be increased according to the need.

Figure 12: Daily Release of Ferrous Gluconate and Ascorbic Acid from the Hydrogel



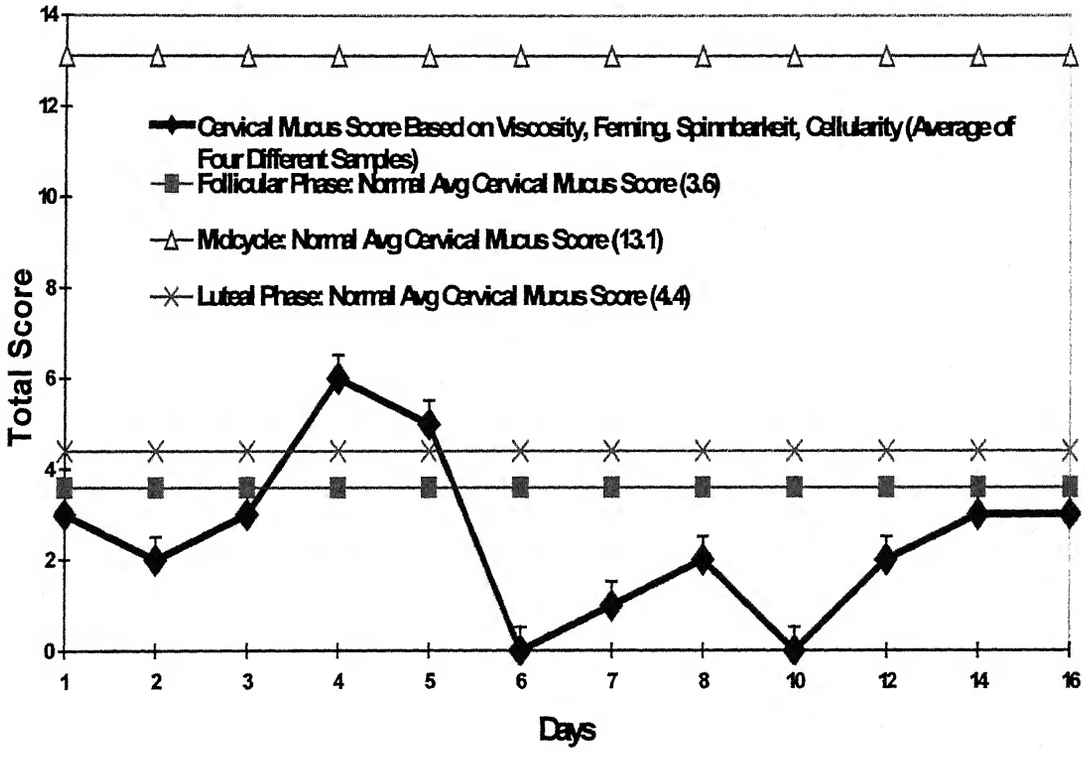
Concentrations are significantly lower than US Pharmacopeia suggested toxic levels (LD-50) ferrous gluconate: 4.5 g/kg body weight

L-ascorbic acid: 1.2 g/kg body weight

1.1.2. Effect of Daily Eluates on Cervical Mucus Score

As shown below, the cervical mucus score was not more than 6 during the entire period of observation, which is far below than the mid-cycle cervical mucus score of approximately 13.1.

Figure 13: Effect of Daily Eluates from the Hydrogel on Cervical Mucus Score



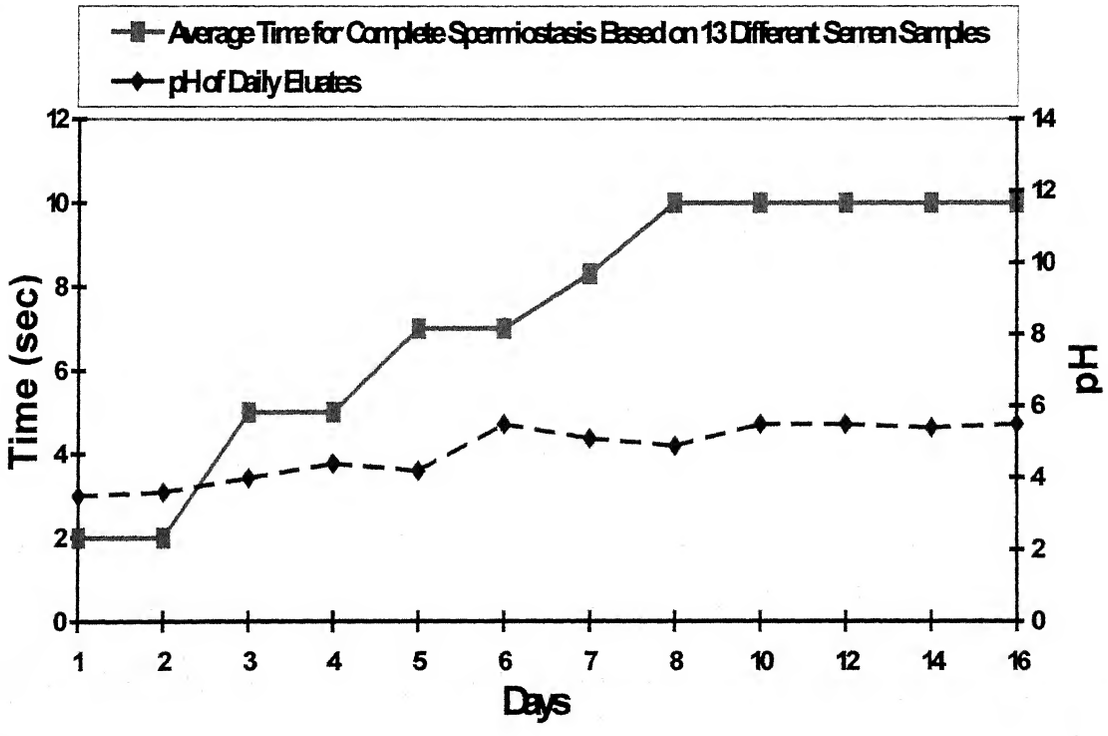
The cervical mucus scoring was conducted according to the WHO guideline.

1.1.3. Spermiostatic Effect and pH of Daily Eluates

Semen samples from 13 different patients with good sperm count and motility was analyzed with 16 samples of eluates. The figure below represents the cumulative observation.

Full spermiostatis occurred within 30 secs. From Day 1 – 16 and the pH of the elaute remained between 3.5 – 5.5

Figure 14: Spermiostatic Effect and pH of Daily Eluates from the Hydrogel



1.1.4. Sperm penetration test

Upon examination of slides prepared with cervical mucus and semen samples, the sperm were observed to be immobile or moving at a sluggish speed as compared to the control in which they penetrated the cervical mucus with normal motility. Not only was sperm movement hindered by the ferrous gluconate, but the ascorbic acid also increased mucus viscosity so that it could not be easily penetrated.

1.1.5. Effect on vaginal flora

Microbiology testing of the vaginal swab treated with eluates indicated no adverse effects of the eluates on the growth of lactobacilus

1.1.6. In vivo studies in estrous female rabbit

Examination under a phase contrast microscope over a heated stage of the post-insemination flush from a rabbit vagina instilled with the hydrogel showed sperm to be immobile. Addition of human tubule fluid (Irvine Scientific, Irvine, CA) failed to reanimate sperm. Insemination and examination of the post-insemination flush repeated 24 hours later produced similar results. The external genitalia of the male and female rabbits appeared to have no inflammatory response. The female rabbits were examined after four weeks as a follow-up and they had not become pregnant. While this observation along with spermiostatic effects in the rabbit are convincing, these results remain to be substantiated by

detection of fertilized eggs or implantation sites to completely determine the efficacy of the gels. Although there is no appropriate model that directly corresponds to human subjects, the rabbit offers a reliable comparison and reinforces the in vitro results.

2. Discussion

Current methods of contraception do not offer sexually active women complete protection. Many contraceptives have associated side effects due to altered hormone levels. The NuvaRing is currently available for use; however, it is a hormonal contraceptive that does not protect against sexually transmitted diseases. There is a need for a non-hormonal, biocompatible contraceptive with anti-infective agents. Such a device would bypass the systemic route in the body, allow for coital independence, and protect its user against unwanted infections. Hydrogels are the optimal choice in drug delivery systems for a variety of reasons. High water content makes them biocompatible. Hydrogels mimic the hydrodynamic properties of cells and tissues, minimizing mechanical and frictional irritation. Hydrogels combine the merits of natural biodegradable polymers like dextran with synthetic biodegradable polymers like PLA into a single entity via chemical cross-linking so that there is no phase separation and thus allow a predictable release of the incorporated biochemical agents. The degradation products

of PGA and PLA are natural metabolites and are readily eliminated by the human body. Dextran was found to affect mucus spinnbarkeit due to the polymerization of the mucous glycoprotein, resulting also in an increase in the viscosity of the cervical mucus. Dextran is hydrophilic, biocompatible and contains 1,6-α-D-glucopyranosyl residues. Each glucose residue has three hydroxyl groups, providing greater flexibility in the formulation of hydrogels. A core-sheath design of the hydrogel was selected to minimize burst effect and maintain sustained release. The core functions as the drug reservoir. The sheaths control the diffusion of liquid into the hydrogel core.

Ferrous gluconate targets the sperm tail and promotes lipid peroxidation. Lipid peroxidation is a type of cellular damage involving the formation of free oxygen radicals, such as superoxide anion, O^2. The radicals are extremely unstable and unfavorable to the lipid bilayer of a cell, resulting in cell damage. Human spermatozoa are enriched with unsaturated fatty acid, and fatty acids are particularly susceptible to lipid peroxidation. The lipid peroxidation process is initiated as an antioxidant defense system, superoxide dismutase. This reaction occurs when lipid peroxides in the bilayer of sperm tails are exposed to ferrous ion resulting in the propagation of lipid peroxidation, which leads to a continuous formation and decomposition of lipid peroxides. Eventually, this causes structural damage, a decline in metabolic activity, and spermiostatic effects on sperm.

L-ascorbic acid acts on the cervical mucus to change it from the open cellular structure found at mid-cycle of the menstrual period to the closed structure in order to form an impenetrable barrier for sperm. Ascorbate acts as an antioxidant by transferring electrons and changing the protein conformation. It reduces the disulfide (-S-S-) bonds of glycoprotein mucopolysaccharides in the cervical mucus, inhibiting sperm motility. As mentioned by Jones and Mann, ascorbate re-reduces Fe^{3+} to Fe^{2+}, which act synergistically. To further ensure that sperm do not survive, Ampholine sustains the pH close to 4.5.

3. Future studies

3.1. Future human studies

The BioRing will need to gain approval from the FDA as an Investigational Device Exemption (IDE) for future human studies. Studies involving tubectomized female volunteers will be performed for 2 puposes, firstly, to study the contraceptive efficacy and, secondly, to monitor any change in the evaginal flora.

3.1.1. Prevention of HIV

The first step in HIV protection would be to incorporate an antiviral agent into the BioRing. The antiviral agent should be safe, non-irritating, and

affordable. Of the 6 currently available group of HIV drugs, we are considering protease inhibitors, specifically, Kaletra (lopinavir/ritonavir).

3.2. Further possible developments to the BioRing

1) The hydrogel will also be encapsulated in rapidly water soluble soft gelatin capsule. Alternatively, the hydrogel precursors will be pumped into empty gelatin capsules first and then allowed to gel by photo-cross-linking as described in preliminary studies. The soft gelatin will dissolve in water within few minutes, leaving the hydrogel ring to biodegrade and release the spermiostatic agents. Alternatively, the hydrogel ring will also be coated with hydroxy-propyl-methyl cellulose a biodegradable and biocompatible compound to provide necessary firmness if required.

2) Another approach will be to use biodegradable Chitosan-Poly (vinyl) alcohol (PVA) hydrogel matrix as an outside sheath or fused with the hydrogel. Chitosan [α (1, 4) 2-amino 2-deoxy β D glycan] is a linear mucopolysaccharide. Chitosan is widely used in drug delivery systems, especially in mucosal membranes. The PVA hydrogel has been used as a material for artificial kidney, skin replacement and for reconstructive joint surgery for synthetic articular cartilage. The PVA was also used as a hydrophilic component added to mixtures of other excipients and drugs.

3) Chitosan: Chitin is a major structural polysaccharide found in invertebrate animals and lower plants. Chitosan [α (1, 4) 2-amino 2-deoxy β D glycan] is the principal deacetylated derivative of chitin. It is a linear mucolysaccharide with a structure similar to glycoasminoglycans. Commercial chitosan is produced by deacetylation of chitin from seashell materials. For industrially produced chitosan, the molecular weight (between 100,000 to 1,200,000) and the degree of deacetylation are the most important quality indices. Chitosan is widely used in pharmaceutical preparations for the development of drug delivery systems because of its availability, biocompatibility, biodegradability, and inexpensiveness. The applications include the nasal, mucosal, ocular, oral, and transdermal delivery of drugs and delivery of other agents such as pesticides, nematocides, and insecticides. The use of chitosan in the development of drug delivery systems is based on experiences with chitosan intragastric tablets. Drugs are dispersed in chitosan-coated drug delivery systems. Drugs dispersed in chitosan have been found to be released at a constant rate. This highlights its potential use as a sustained release matrix. Chitosan also exhibits significant mucoadhesive properties.

Bodmeier et al prepared drug-entrapped microparticles encapsulated in chitosan beads by ionotropic gelation of chitosan in tripolyphosphate solution for controlled release. After eluting with 0.1

N HCl, the chitosan beads disintegrated and released the microparticles. The disintegration time appeared to be a function of the polysaccharide viscosity, gelation time, and drying methods. In another report, the model drug prednisolone was loaded in three types of chitosan films: monolayer, double layer, and N-acetylated chitosan double layer for retarded release studies. The drug release was found to be the slowest when using N-acetylated film.

The amino and hydroxyl groups on the chitosan provide places for physical or chemical linkages and modification. Chitosan therefore has been widely used as a carrier for enzyme immobilization. The immobilization often involves a cross-linking step using glutaraldhyde. However, other cross-liners such as glyceraldehydes can also be used.

4) Poly (vinyl) alcohol (PVA) is a widely used polymer noted for its excellent mechanical properties. It is also biodegraded under proper conditions (44). Since the monomer of vinyl alcohol does not exist in a stable form, the commercially available PVA is produced by first polymerizing vinyl acetate (PVAc) and then hydrolyzing it. The commercial PVA is a mixture of different types of stereoregular PVA structures such as isostatic, syndiotactic, and atactic. The stereoregularity and the chemical and physical properties of PVA are dependent on its method of preparation.

The PVA hydrogels are the basic materials m a variety of biomedical applications. They have been studied extensively as potential biomaterials for contact with blood both in their native form and as heparinized materials. For example, a prototype heparin-PVA hydrogel was formed using glutaraldehyde as the cross-linker. The gel was reported to be transparent, and behaved like an ideal vulcanized rubber with excellent elasticity. The PVA hydrogel has been used as a material for artificial kidneys and skin replacement. PVA was also tested for using reconstructive joint surgery as synthetic articular cartilage was also tested. A spongy cross-linked PVA hydrogel was used for augmentation for mamoplasty due to the elasticity and good dynamic mechanical properties. The PVA was also used as a hydrophilic component added to mixtures of other excipients and drugs to compress tablets. The release of small and large molecular weight solutes, including phenyl propanolamine, KCL and bovine serum albumin from these tablets were examined. Swelling-linked double layer PVA beads were also developed for slow release of acetaminophen and procyphylline at 37°C

5) Chitosan-PVA Hydrogel: We have prepared and characterized chitosan-PVA hydrogels using glutaraldehyde as the cross-liner. This is a pH-sensitive, semi-interpenetrating network (semi-IPN) hydrogel. This hydrogel is strong and pliable. The compressive strength increases with the PVA content. The pH-sensitivity is maximum at

about 3.5 in terms of the volume increase. The controlled-release properties were studied using vitamin B as the model drug. The release rate is pH dependent (highest at pH 3.5). This study indicated that by varying the PVA content and cross-linker concentration the release profile can be altered significantly.

6) Proposed Chitosan-PVA Gel Matrix: Chitosan-PVA hydrogel will be prepared using different chitosan: PVA molar ratios (1:1; 1:2.5; 1:5; and 1:10) and using different glutaraldehyde concentrations (15, 25, 50, and 75 μM in the gel). The drug ferrous gluconate will be encapsulated during gel preparation.

- Form the hydrogel as a ring (or any required shape). This is probably not the best unless combined with additional barriers, such as gelatin or calcium alginate or HPMC.
- Form hydrogel as microbeads of different diameters and with different levels of drug loading housed in an annulus pouch (made of the sheath materials). The idea is that different gel beads will have different release rates with a sustained composite delivery rate. The layered pouch can improve sustained delivery.
- As explained in b) above, but the hydrogels are coated with some additional barriers, as necessary such as calcium alginate.

4. Bibliography

Roy S, Mishell D. Status of research and development of vaginal contraceptive rings as fertility control method in the female. Research Frontiers in Fertility Regulation. 1983:2(4): 1-10.

Network, Family Health International, 2000.

U.S. Patent # 5,667,492. (Bologna W). Use and composition of anti-sexually transmitted diseases formulation.

U.S. Patent 5, 595, 980 (Brode G), Contraceptive compositions (Anti-HIV effect).

U.S. Patent 4,959,216 (Daunter B), Contraceptive methods and delivery thereof.

Shoham J, et al. Influence of different copper wires on human sperm penetration into bovine cervical mucus, in vitro. Contraception. 1987; 36(3):327-34.

U.S. Patent # 3,950,366 (Diveley W). Tetrasubstituted dithiobiuretics.

U.S. Patent # 5,224, 493 (Sawan S). Contraceptive intracervical device and noel non-systemic agents for the prevention of conception and disease.

U.S. Patent # 5,617,877. (Moench T). Method for acidifying an ejaculate of semen.

Olmsted SS, Dubin NH, Cone RA, Moench TR. The rate at which human sperm are immobilized and killed by mild acidity. Fertility and Sterility. 2000; 73:687-693.

Kim SH. Polysaccharide-based hydrogels as drug control release devices. PhD Thesis, Cornell University, Ithaca, New York, July, 1999.

Zhang Y. Synthesis, characterization and biomedical applications of new classes of biodegradable hydrogels made of polysaccharides and synthetic biodegradable polymers. PhD Thesis. Cornell University, Ithaca, New York. May 2000.

Kim SH, Won CY, Chu CC. Synthesis and characterization of dextran-maleic acid based hydrogel. J Biomed Mater Res. (In Press).

Kim SH, Won CY, Chu CC. Synthesis and characterization of dextran-methacrylate and its structure study by SEM. J Biomed Mater Res. 2000; 49(4):517.

Zhang Y, Won CY, Chu CC. Synthesis and characterization of novel biodegradable IPN hydrogels having both hydrophobic and hydrophilic components with controlled swelling properties. J Polymer Chemistry. 1999;37:4554-4569.

Zhang Y, Won CY, Chu CC. Synthesis and characterization of biodegradable hydrogels having poly-lactide and dextran components with controlled swelling properties. J Polymer Chemistry (In Press).

Kim SH, Chu CC. In vitro drug release behavior of deKtran-methacrylate hydrogel using anticancer antibiotics, doxorubicin and other model compounds. J Control Release (Pending).

Kim SH, Won CY, Chu CC. Dextran maleic acid monoesters and hydrogels therefrom. U.S. Patent filed on Aug 31, 1998.

Park K, Shalaby WSW, Park H. Biodegradable hydrogels for drug delivery. Technomic Lancaster, PA. 1993.

Won CY, Zhang Y, Chu CC. Hydrogel forming system with hydrophobic hydrophilic components. U.S. Patent filed on April 19, 1999.

Harrison P, Rosenfeld A. Contraceptive research and development. National Academy of Sciences. National Academy Press. 1996:1-27.

Hong CY et al. Effect of lipid peroxidation on beating frequency of human sperm tail. Andrologia. 1993; 26:61-65.

Aitken RJ, Harris D, Buckingham D, Relationship between iron-catalyzed lipid peroxidation potential and human sperm function. a Reproduction and Fertility. 1993; 98:257-265.

Calamera JC et al. Effect of lipid peroxidation upon human spermatic adenosinetriphosphate (ATP). Relationship with motility, velocity and linearity of the spermatozoa. Andrologia. 1988; 21(1):48-49.

Jones R, Mann T. Lipid peroxidation in spermatozoa. Proc Roy Soc. London. 1973; 184:103-107.

Jones R, Mann T. Lipid peroxides in spermatozoa formation, role of plasmalogen and physiological significance. Proc Roy Soc. London. 1976; 193:317-333.

Jones R, Mann T. Damage to ram spennatozoaly peroxidation of endogenous phospholipids. J Reproel Ferti. 1977; 50:261-268.

Jones R, Mann T, Sherins RJ. Adverse effects of peroxidized lipids on human spermatozoa. Proc Roy Soc. London. 1978; B201:413-417.

WHO Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 1999;Ch. 5:51-59.

Nguyen NY, Chrambach A. Stabilization of pH gradients formed by ampholines. Biochemistry. 1977; 82(1):226-35.

Bedford JM. The rate of sperm passage into the cervix after coitus in the rabbit. J Reprod Fertil. 1971; 25:211-218.

Bedford JM. Techniques and criteria used in the study of fertilization. In: Daniel J. Jr. (ed) Methods in Mammalian Embryology. WHO Freeman & Co. NY. 1971:37-63.

U.S. Patent # 6,262,034. (Duramed Pharmaceuticals, Inc). Issue date 04 07 2000 Sustained release formulation containing three types of polymers. (Hydroxyprpyl methycellulose. Zhang, Guohua, Pinnamaraju, Prasad, Ali, Muhammad

Chadarack WM, Brusek DA, Santomauro AP, Fazekas G. Experimental studies on synthetic substitutes for skin and their use in the treatment of burns. Ann Surg. 1962; 155:127.

Movshovich IA, Roitberg GI. Polymeric materials in construction of endoprotheses of the pelvic-femoral joint. Med Tekken.1974;5:15-19.

Inouye K, Machida Y, Sannan T, Nagai T. Buoyant sustained release tablets based on chitosan. Drug Des Deliv. 1988; 4(1):55-67.

Sheth PR, Tossourman J. The hydrodynamically balanced system for oral use. Drug Dev Ind Pharm. 1984; 10:313-339.

Chein YW. Potential developments and new approaches in oral controlled release drug delivery system. Drug Dev Ind Pharm. 1983; 9:1291-1330.

Kawashimay Y, Kasai A, Takenaka H, Lin SY. The effects of thickness and hardness of the coating film. on drug release rate of theophylline granules coated with chitosan as drug carriers. Chem Pharrn Bull. 1981; 29:3067-3069.

Lehr CM, Bouwstra JA, Schacht EH, Junginger HE. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. Int J Pharm. 1992; 78:43-48.

Bodmeier R, Chen H, Paeratakul O. Preparation and evaluation of drug-containing polymeric nanosuspensions. Pharm Res. 1989; 6(5):413-417.

Kanka M, Katayama H, Tsuzuki S, Kurimoto H. Application of chitin and chitosan to pharmaceutical preparations 1: Film preparation and in vitro evaluation. Chem Pharm Bull. 1989; 37(2):523-525.

Takasu A, Aoi K, Tsucyia M, Okada M. New chitin-based polymer hybrid 4: Soil burial degradation behavior of poly (vinyl alcohol)/chitin derivative miscible blends. J Appl Polym Sci, 1999; 73:1171-1179.

45.1Zees TD, Guy CL, Coburn RJ. The use of inflatable breast implants. Plast Reconstr Surg. 1973; 52:609.

Korsmeyer RW, Peppas NA. Effect of the morphology of hydrophilic polymeric matrices on its diffusion and release of water soluble drugs. J Membrane Sci. 1981; 9:211.

Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of solute release from porous hydrophilic polymers. 7nt J Pharm.1983;15:25.

Kim J, Lee Y, Kim K. Properties and swelling characteristics of cross-linked polY(vinyl alcohol)/chitosan blend membrane. J App Polym Sci. 1992; 45:1711-1717.

Gunasekaran S, Wang T. Properties of a pH-sensitive smart hydrogel. Proceedings of 25' Annual Northeast Bioengineering Conference, Hartford CT. April 8-9, 1999.

Wang T, Gunasekaran S. Gelation and controlled release of a pH-sensitive, biodegradable hydrogel. Abstract No.78D-7. Book of Abstracts. Ift Annual Meeting, Dallas, TX. June 10-14, 2000.

Budahari V, O'Neill M, Smith A, Heckelman P. The Merck Index: an encyclopedia of chemicals, drugs and biologicals. 1996:699,1037.

Welcher F. Standard Methods of Chemical Analysis. Phenanthroline.1961:910-911.

Roe JH. Standard Methods of Clinical Chemistry. Seligson D, ed. New York: Academia Press. 1961; 3:35.

Kharadze D, Kirmelashvilli L, Medzmariashvilli N, et al. Amino acid based bioanalogus polymers: Synthesis and a-chymotrypsinolysis study of regular poly(ester amides) based on phenylalanine, diols and terephthalic acid. Polymer Science. 1999;Ser. A,41(9):883-890.

Katsarava R, Beridze V, Arabuli N, Kharadze D, Chu CC, Won CY. Amino acid based bioanalogous polymers: Synthesis and study of regular poly(ester amide)s based on bis(a-amino acid)a,co-alkylene diesters and aliphatic dicarboxlic acids. J Polym Sca. Polymer Chemistry ed. 1999; 37:391-407.

Sawayanagi Y, Nambu N, Nagai T. Directly compressed tablets containing chitin and chitosan in addition to lactose or potato starch. Chem Pharm Bull. 1982; 30:2935-2940.

Mulders TMT, Dieben TOM, Coelingh B, Herjan JT. Ovarian function with a novel combined contraceptive vaginal ring. Hum Reprod 2002; 17:2594-9.

Van der Spoel AC, Jeyakumar M, Butters TD, Charlton HM, Moore Dwek RA, Platt FM. Reversible infertility in male mice after oral administration of alkylated imino sugars: a nonhormonal approach to male contraception. Proc Natl Acad Sci 2002; 99:17173-8.

Superville D. FDA Strengthens HRT Warning. The Associated Press, 2003 Jan 8, Sect A: 6.

Moreno V, Bosch FX, Munoz N, et al. Long-term use of oral contraceptives and a high number of children increase the risk of cervical cancer in women with HPV infection. Lancet 2002; 359:1085-92.

Smith JS, Green J, Berrington de Gonzalez A, et al. Cervical cancer and use of hormonal contraceptives: a systematic review. Lancet 2003; 361:1159-67.

World Health Organization. Cervical Cancer, Oral Contraceptives and Parity. The Organization, 2003.

Wells E. Risk of cervical cancer: Linked to duration of oral contraceptive use? Medica.de [serial online] 2003 July 4; Available from URL: http://www4medica.de/cipp/md_medica/custom/pub/content,lang,2/tickets,g_a_s_t/oid,9464/local_lang, 2

Harrison P, Rosenfield A. Contraceptive research and development. Washington D.C.: National Academy Press, 1996. p. 1-27.

Daunter B, inventor. University of Queensland, assignee. Contraceptive methods and delivery systems thereof. US patent 4,959,216. 1990 Sept 25.

Moench TR, Cone RA, inventors. Method for acidifying an ejaculate of semen. US patent 5,617,877. 1997 Apr 8.

Olmsted SS, Dubin NH, Cone RA, Moench TR. The rate at which human sperm are immobilized and killed by mild acidity. Fertil Steril 2000; 73:687-93.

Shoham J, Lidor A, Megory E, et al. Influence of different copper wires on human sperm penetration into bovine cervical mucus, in vitro. Contraception 1987; 36:327-34

Diveley WR, inventor. Hercules Incorporated, assignee. Metal salts of 1,1,5,5-tetrasubstitued dithiobiurets. US patent 3,950,366. 1976 Apr 13.

Brode GL, Doncel GF, Gabelnick HL, Kreeger RL, Salensky GA, inventors. Medical College of Hampton Roads, Biomaterials Corporation, assignee. Contraceptive compositions. US patent 5,595,980. 1997 Jan 21.

Zhang X, Won CY, Chu CC. Synthesis and characterization of novel biodegradable IPN hydrogels having both hydrophobic and hydrophilic components with controlled swelling properties. J Poly Chem 1999; 37:4554-69.

Park K, Shalaby SW, Park H. Biodegradable hydrogels for drug delivery. Lancaster (PA): Technomic; 1993.

Roy S, Mishell D. Status of research and development of vaginal contraceptive rings as fertility control method in the female. Res Front Fertil Reg 1983; 2:1-10.

Nguyen NY, Chramach A. Stabilization of pH gradients formed by ampholines. Biochem 1977; 82(Pt 1): 226-35.

Hong CY, Lee MF, Lai LF, et al. Effect of lipid peroxidation on beating frequency of human sperm tail. Andrologia 1994; 26:61-5.

Aiken RJ, Harris D, Buckingham D. Relationship between iron-catalyzed lipid peroxidation potential and human sperm function. J Reprod Fertil 1993; 98:257-65.

Calamera JC, Giovenco P, Quiros MC, et al. Effect of lipid peroxidation upon human spermatic adenosinetriphosphate (ATP). Relationship with

motility, velocity and linearity of the spermatozoa. Andrologia 1989; 21:48-54.

Jones R, Mann T. Lipid peroxidation in spermatozoa. Proc Roy Soc London 1973; 184:103-7.

Jones R, Mann T. Lipid peroxides in spermatozoa formation, role of plasmologen and physiological significance. Proc Roy Soc London 1976; 193:317-33.

Jones R, Mann T. Damage to ram spermatozoa formation, role of plasmalogen and physiological significance. J Reprod Fertil 1977; 50:261-8.

Jones R, Mann T, Sherins RJ. Adverse effects of peroxidized lipids on human spermatazoa. Proc Roy Soc London 1978; 201:413-7.

World Health Organization. Sperm-cervical mucus interaction. In: WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction, 4th ed. New York: Cambridge University Press, 1999. p. 51-9.

Bedford JM. The rate of sperm passage into the cervix after coitus in the rabbit. J Reprod Fertil 1971; 25:211-8.

The Merck Index: an encyclopedia of chemicals, drugs and biologicals. New Jersey: Merck; 1996. Apraxia; p. 699,1037.

Bedford JM. Techniques and criteria used in the study of fertilization. In: Daniel J Jr, editor. Methods in mammalian embryology. New York: WHO Freeman, 1971. p. 37-63.

Welcher F. Phenanthroline. In: Furman NH, editor. Standard methods of chemical analysis, 6th ed. New Jersey: Van Nostrand Company, 1962. p. 910-1.

Roe JH. L-ascorbic acid. In: Seligson D, editor. Standard methods of clinical chemistry. New York: Academia Press, 1961. p. 33-5.

Conclusion

Contraception prevents unwanted pregnancies and provides better family planning and health care. Convenience, safety, efficacy, and cost as well as the quality of life are usually the concerns in choosing a contraceptive, and these very factors motivate the development of newer and better contraceptives. There is a pressing need to develop a non-hormonal, biocompatible, non-invasive, cost-effective, biodegradable, and convenient device to prevent pregnancy and infection without interfering with sexual relations. The success rate of a contraceptive depends not only upon the efficacy of the contraceptive method, but also upon the users' preference, reversibility, convenience, and compliance. Besides pregnancy, sexual relations can also result in infection. It is thus beneficial that the design of newer contraceptive devices should also consider the option of protecting women not only against pregnancy but also against transmission of sexually transmitted diseases (STDs). The new contraceptive devices could be free of hormones and toxic compounds and should allow women to insert by themselves in

conjunction with normal management of their menstrual cycle month after month, thus enhancing the quality of life. One of the recommendations made by the Committee on Contraceptive Research and Development in 1996 was to identify agents that are spermiostatic rather than spermicidal, modify mucus secretions from cervical epithelial cells to prevent sperm penetration and are anti-microbial and anti-viral. With current interest in the delivery of steroids as a contraception method and during post-menopause via non-biodegradable, hormonal intravaginal rings, it is innovative to create a biodegradable, non-hormonal and biocompatible intravaginal ring that acts locally and avoids a systemic route to deliver contraceptive agents. The new device could also carry anti-STD agents, since N9 spermicides are not available. Many women depend on them. Thus the development of BioRing is timely and has innovation and significance for women's' health care.

Studies presented lead to the conclusion that biodegradable hydrogels impregnated with L-ascorbic acid, ferrous gluconate and Ampholine could lead to the development of a non-hormonal and biocompatible intravaginal contraceptive device. However, further improvement of the hydrogel and comprehensive pre-clinical studies are necessary prior to use in women. The hydrogel also has a potential as a delivery system for anti-STD, and anti-HIV agents as well as drugs to treat pelvic diseases. Such devices will act locally and minimize adverse systemic effects. The hydrogel composition could be chosen to last for the period, up to one

month, as required by the user. Further, the gel could be used additionally as a spermicide inside a condom.

The contraceptive ring in development has taken into account many considerations and strives to deliver quality and effective protection from pregnancy, while not compromising comfort, ease, and peace of mind and body.

In view of the lack of toxicity and biocompatibility of the components used in the intravaginal device (ferrous gluconate, ascorbic acid, and polyamino-polycarboxylic acid), which have already been approved individually by the FDA, no additional safety data in humans is considered necessary. It will thus not be necessary to go through the IND or NDA process. The total month's supply of iron in the ring is less than what is proven to be toxic in humans. Thus even if all the iron was somehow absorbed immediately from the ring, rather than over a 28-day period, the amount would still not be harmful. Moreover, the iron will be useful to these women of child-bearing age because they lose iron monthly through menstruation. Furthermore, the vitamin C in the ring will help the woman to absorb this beneficial iron.

The biodegradable, biocompatible, noninvasive hydrogel ring can also have uses independent of non-hormonal contraception. Filled with antimicrobicides, the ring could be used solely as protection against

STDs and/or HIV. In this capacity, the ring could be created for 1-day, 1-week, or month-to-month use.